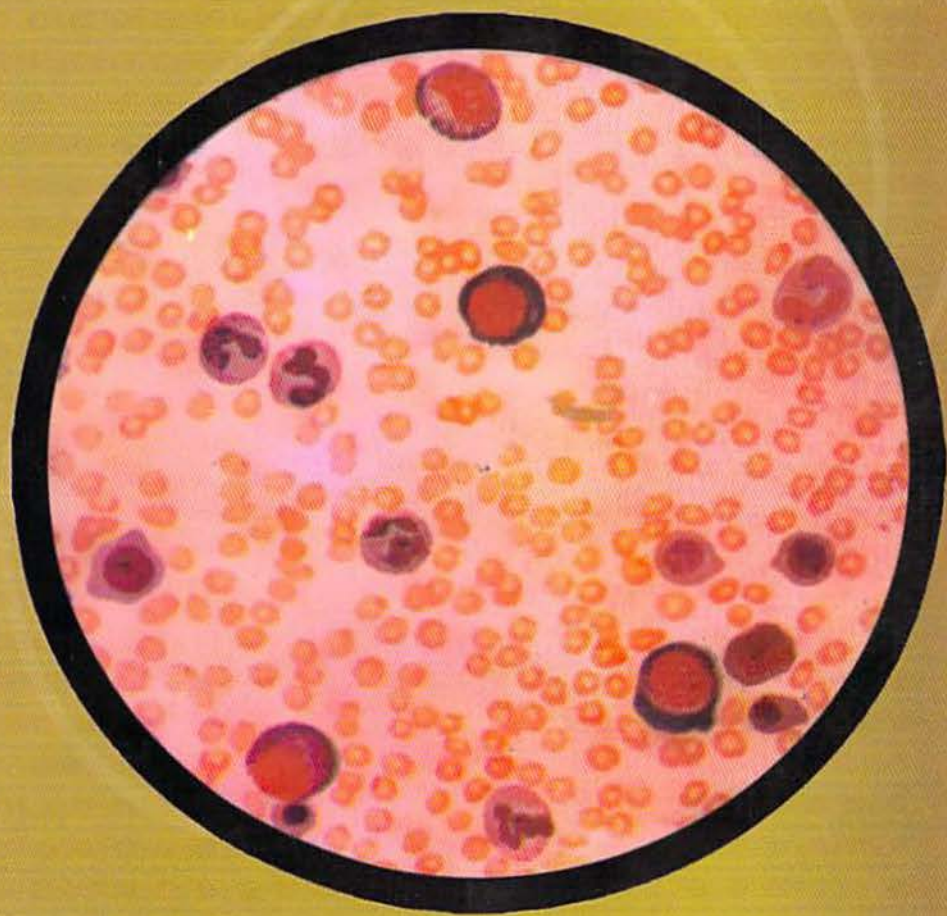


Human

PHYSIOLOGY

for medical students



BLOOD AND BODY FLUIDS

Magdi Sabry M.D.

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HUMAN
PHYSIOLOGY
FOR MEDICAL STUDENTS

BLOOD AND BODY FLUIDS

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DEDICATED TO

MY TEACHERS THROUGHOUT MY LIFE, particularly the first 2 teachers, my late parents.

MY FAITHFUL LATE WIFE, the light that disappeared from life but is everlasting inside me.

MY SONS, SHERIF, AMR AND ESSAM, AND THEIR WIVES, their love gives me hope and interest in life and makes their happiness my chief goal.

MY GRANDCHILDREN, AHMED, NOURHAN and SAMA SHERIF, YASMIN and MOHAMMED AMR, AND AYA and ALY ESSAM, the beautiful young angels that have added a new kind of happiness to my life.

Preface

With recent advances in the field of human physiology, it has become urgent to provide an up to date review on the subject.

This book is provided to help medical students in understanding modern human physiology. It presents the whole subject in a brief, comprehensive and up to date form.

Great effort was done to perfect such a vast subject in an easily understandable expression and in a such reasonable bulk. It includes as much simplified and clear illustrations as possible, and to maintain simplicity, they have been presented in a diagrammatic form, photographs were greatly excluded.

The major part of my gratitude should be given to all who taught me, and to my late wife and sons who, patiently, supported me during preparation of this book.

Any suggestions, remarks or criticism will be greatly welcomed, heartily appreciated and very much considered.

I hope this book will be a real help to undergraduate medical students as well as to postgraduates and candidates of higher degrees, in the field of human physiology.

MAGDI SABRY, 1987

Second edition : 1993

Third edition : 1997

Fourth edition : 2001

Preface to the fifth edition

This new edition is original both in shape and contents. All chapters have been extensively revised and updated. More figures and illustrations were introduced, and recent data were added elsewhere specially about the defence mechanisms of the body, and most subjects have been completely rewritten.

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THE BODY FLUIDS

THE TOTAL BODY WATER (TBW) AND ITS DISTRIBUTION

The TBW is normally about 60 % of the body weight in young adult males, 51 % in young adult females, and 45 % in obese persons (because the body fat is relatively free of water).

Therefore, in a young adult male weighing 70 Kg, the TBW is normally about 42 litres, and it is distributed as follows (figure 1) :

(1) **Intracellular fluid (ICF)** : This constitutes about 2/3 of the TBW i.e. about 28 litres (40 % of the body weight).

(2) **Extracellular fluid (ECF)** : This constitutes about 1/3 of the TBW i.e. about 14 litres (20 % of the body weight), and it includes the following subdivisions :

(a) **Intravascular fluid (i.e. the plasma)** : This is normally about 1/4 the ECF volume i.e. about 3.5 litres (5 % of the body weight).

(b) **Extravascular fluid** : This is normally about 3/4 the ECF volume i.e. about 10.5 litres (15 % of the body weight), and it includes the

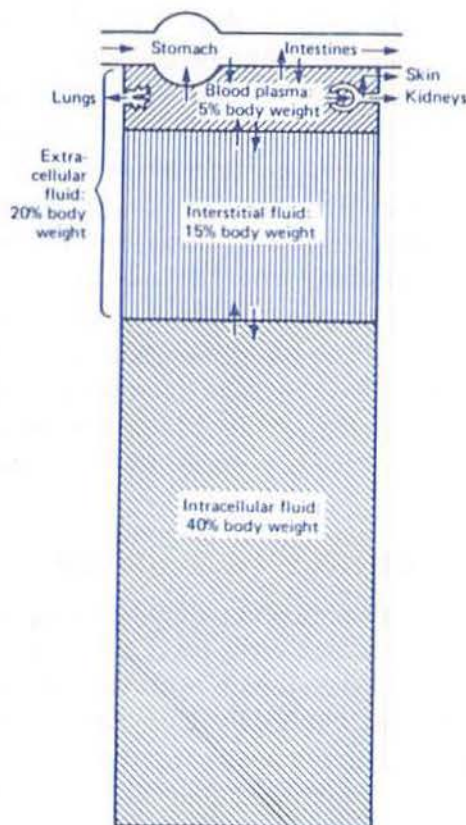


Figure 1 : Distribution of water in the body.

interstitial fluid (= tissue fluid) + transcellular fluids (cerebrospinal and intraocular fluids, and the fluids in the joints, pleura, peritoneum, etc).

FUNCTIONS OF THE BODY WATER

- (1) It is a medium for the chemical and enzymatic reactions.
- (2) It is a medium for the physical processes e.g. diffusion and filtration.
- (3) It is an ionizing medium (regulating pH and body fluids osmolality).
- (4) It regulates the body temperature through heat absorption and distribution and evaporation (refer to metabolism).
- (5) It is a lubricant in the joints and the potential spaces (e.g. the pleura).
- (6) It is a refractive medium in the eye.
- (7) The cerebrospinal fluid is a mechanical buffer that protects the brain.
- (8) It is the medium for exchange of O_2 and CO_2 in the lungs and tissues.

COMPOSITION OF THE ECF and ICF

(A) **ECF** : The composition of the ECF is almost the same elsewhere, except for the *protein concentration which is much higher in the plasma* than in the interstitial fluid (about 17 mEq/litre and 5 mEq/litre respectively). The *main cation* is Na^+ (about 142 mEq/litre) while the *main anion* is Cl^- (about 103 mEq/litre). *Other cations* include K^+ (about 4 mEq/litre) and small amount of Ca^{2+} and Mg^{2+} while *other anions* include HCO_3^- (about 28 mEq/litre), proteins and small amounts of HPO_4^{2-} and SO_4^{2-} . The ECF also contains *non-electrolytes* such as glucose, cholesterol, urea, uric acid, creatinine, bile pigments and phospholipids. Its *pH* is about 7.4 and its *osmolality* is about 300 mOsm/litre.

(B) **ICF** : The *main cations* include K^+ and Mg^{2+} (about 140 mEq/litre and 58 mEq/litre respectively) together with a small amount of Na^+ and very little Ca^{2+} . The *main anions* include HPO_4^{2-} and protein (about 75 mEq/litre and 40-45 mEq/litre respectively) together with small amounts of Cl^- , HCO_3^- and SO_4^{2-} . Its *pH* is less than that of the ECF (about 7) due to its low HCO_3^- content, while its *osmolality* is equal to that of the EC.

FLUID EXCHANGE BETWEEN ECF and ICF

Normally, the cell membranes are highly permeable to water, so fluid exchanges continuously across them resulting in an equal osmolality in both the ECF and ICF. This condition is maintained as follows :

(1) If a *hypertonic NaCl solution* is i.v. injected, the ECF osmolality increases. This causes water transfer from the ICF to the ECF till an osmotic equilibrium is established and the osmolality of both fluids become equal again. As a result, the ICF volume decreases, so *the cells are dehydrated and shrink* (in case of R.B.Cs, this is called *crenation*).

(2) If a *hypotonic NaCl solution* (or water) is i.v. injected, the ECF osmolality decreases. This causes water transfer from the ECF to the ICF till an osmotic equilibrium is established and the osmolality of both fluids become equal again. As a result, the ICF volume increases, so *the cells are swollen and may rupture* (in case of R.B.Cs, this is called *hemolysis*).

****** Injection of *isotonic solutions* mainly increase the ECF volume (resulting in edema), but they do not affect the osmolality of the body fluids.

MEASUREMENT OF THE TBW

This is carried out by using *the indicator dilution principle* as follows :

(1) A known amount of an indicator substance is i.v. injected. The used indicator *should penetrate the cell membranes* to be dispersed in both the ECF and ICF. The commonly used indicators are (a) *Deuterium oxide* ($= D_2O$ or heavy water) (b) *Tritium oxide* (3H_2O) (c) *Aminopyrine*.

(2) A sufficient time is allowed for complete distribution of the indicator in the TBW, then its concentration in the plasma is determined.

(3) The volume of distribution (i.e. TBW volume) is calculated by dividing the injected amount of the indicator by its concentration in the plasma.

MEASUREMENT OF THE ECF VOLUME

The ECF volume is also measured by using the *indicator dilution principle*. However, the indicator used *should not penetrate the cell membranes (to be dispersed in the ECF only)*. The common indicators used for this purpose are *inulin, sucrose and mannitol*.

MEASUREMENT OF THE ICF VOLUME

The ICF volume *cannot be measured directly*. The TBW and ECF volumes should be measured first, and the ICF volume is then calculated by *subtracting the ECF volume from the TBW volume*.

****** The *interstitial fluid volume cannot also be measured directly*. The ECF and plasma volumes should be measured first, and the interstitial fluid volume is then calculated by *subtracting the plasma volume from the ECF volume*.

WATER BALANCE

This is the balance between the daily amounts of *water gain and water loss*. Under normal conditions, *both are equal (2300 ml/day each)* so that the body is in a *normal water balance*, and the TBW is kept constant.

WATER GAIN

This is normally about *2300 ml/day* and is derived from 2 sources :

(1) **Exogenous water** (by the oral route) : This is the *main source* of water gain. It averages *2000 ml/day*, and it includes the volumes of :

- (a) Water and other fluids that are drunk (about *1400 ml/day*).
- (b) Water present in the *eaten food* e. g. water in meat, vegetables and bread (about *600 ml/day*).
- (2) **Endogenous water** (which is formed inside the body as a result of oxidation of H_2 in the foodstuffs). It is normally about *300 ml/day*.

WATER LOSS

In the comfortable zone of atmospheric temperature (about $20^\circ C$), young adult individuals normally lose about *2300 ml of water/day* as follows :

- (a) *1400 ml in the urine* (refer to kidney).
- (b) *100 ml in the feces*.
- (c) *350 ml by evaporation from the respiratory tract*.
- (d) *450 ml from the skin* (mostly as *insensible perspiration* i.e. by non-sensed diffusion and evaporation through the skin).

Water loss through *the skin and respiratory tract* is thus commonly called the *insensible water loss*.

CONTROL OF WATER BALANCE

(A) CONTROL OF WATER GAIN

The amount of water gain is controlled mainly by **the thirst sensation**. Thirst is the *conscious desire for water* that urges the individual to drink. It is produced due to stimulation of a special *thirst centre located in the anterior part of the hypothalamus* (= **central mechanism of thirst**). This occurs if

- (a) The ECF or plasma volumes are decreased (= *hypovolemia*), in which the thirst centre is stimulated by *angiotensin II* and *certain cardiovascular reflexes* (refer to kidney).
- (b) The osmolality (or tonicity) of these fluids is increased (= *hypertonicity*), in which the thirst centre is stimulated *directly*.

In addition, the *decreased salivary secretion* that occurs in cases of hypovolemia causes a sensation of *dryness of the buccal cavity and pharynx* which also urges the individual to drink. This is sometimes called the **peripheral mechanism of thirst**.

(B) CONTROL OF WATER LOSS

The amount of water loss is controlled mainly by adjusting the urine volume, and this is largely determined by the **antidiuretic hormone** (= **ADH or vasopressin**). This hormone is released from the *posterior pituitary gland* and it *increases water reabsorption from the distal segments of the renal tubules of the kidneys* (refer to endocrine glands).

The release of ADH is *stimulated in cases of hypovolemia and hypertonicity* (resulting in increased water retention in the body and decreased water excretion in the urine). On the other hand, *its release is inhibited in cases of hypervolemia and hypotonicity* (resulting in decreased water retention in the body and increased water excretion in the urine).

DISORDERS OF WATER BALANCE

(A) DEHYDRATION

This is a condition of *negative water balance* that occurs when the amount of *water loss exceeds that of water gain* e.g. due to excessive sweating, vomiting or diarrhea and in cases of prolonged water deprivation.

MANIFESTATIONS (SYMPTOMS)

- (1) Cold, pale, dry and wrinkled skin.
- (2) Sunken eyes and depressed fontanelles of the skull (in infants).
- (3) Loss of body weight and muscle weakness together with rapid fatigue.
- (4) Marked thirst and dry mouth (see above).
- (5) Rise of the body temperature (due to sluggish blood flow in the skin).
- (6) Hypotension, weak pulse and circulatory failure (in severe cases).
- (7) Increased plasma proteins concentration and elevation of both the serum Na^+ level and the hematocrit value (due to blood concentration).
- (8) Oliguria or anuria (which may lead to acidosis and uremia).
- (9) Exhaustion of the nerve cells leading initially to excitation followed by delirium then coma and finally death occurs if 20% of the TBW is lost.

**** Infants and children are more vulnerable to develop dehydration** than adults because in the young individuals (a) The ECF / ICF ratio is higher than in adults (b) The kidneys are less able to retain water (c) The daily insensible water loss is greater than in adults.

CORRECTION

(1) **Role of the body** : As a result of hypovolemia, the *release of ADH is increased and the thirst centre is stimulated* (specially if associated with hypertonicity of the plasma). This leads to water retention in the body and excessive ingestion of water (see above).

(2) **Treatment** : Giving the patient fluids either orally (e.g. fruit juices) or i.v. (e.g. *isotonic saline or glucose solutions*).

(B) HYDRATION

This is a condition of *positive water balance* that occurs when the amount of *water gain exceeds that of water loss* e.g. due to *excessive secretion of ADH* and when a dehydrated person drinks *excess water without salt*.

MANIFESTATIONS (SYMPTOMS)

- (1) Generalized edema (with pulmonary edema in severe cases).
- (2) Excessive salivation with nausea and vomiting.
- (3) Decreased plasma osmolality, hematocrit value and Na^+ concentration.
- (4) Signs of *water intoxication* (due to swelling of the nerve cells) which include asthenia, tremors and ataxia followed by convulsions, confusion and drowsiness and finally coma and death.

CORRECTION

(1) **Role of the body** : As a result of hypervolemia and plasma hypotonicity, the *release of ADH is decreased and the thirst centre is not stimulated*. This leads to decreased water excretion in the body, increased water excretion in the urine and minimal ingestion of water (see above).

(2) **Treatment** : Giving the patient (a) Hypertonic saline solutions i.v. (to prevent water intoxication by increasing the plasma osmolality, which decreases the brain edema) (b) Diuretic drugs. The TBW can also be decreased by dialysis.

CHAPTER 2

BLOOD COMPOSITION, PROPERTIES, FUNCTIONS and VOLUME

COMPOSITION OF THE BLOOD

Blood consists of cells suspended in a clear yellowish fluid called the plasma. The cells constitute 40-45 % of the blood volume and include :

(1) **Red blood cells or corpuscles (R.B.Cs) or erythrocytes :** Normally, there are about **5 million R.B.Cs per mm^3** . When they are decreased, the condition is called ***anemia***, and when they are increased, the condition is called ***polycythemia***.

(2) **White blood cells or corpuscles (W.B.Cs) or leukocytes :** Normally, there are **4000-11000 W.B.Cs per mm^3** . When they are decreased, the condition is called ***leukopenia***, and when they are increased, the condition is called ***leukocytosis***.

(3) **Platelets or thrombocytes :** Normally, there are about **300000 platelets per mm^3** . When they are decreased, the condition is called ***thrombocytopenia***, and when they are increased, the condition is called ***thrombocytosis***.

THE PLASMA

The plasma constitutes 55-60 % of the total blood volume, and it consists of water (90 %) and dissolved solutes (10 %). The latter include :

(a) **Organic substances :** Plasma proteins (7.1%), lipids, hormones, enzymes, nutrients and waste products (2 %).

(b) **Inorganic substances (0.9 %)**, which include the various electrolytes e.g. Na^+ , K^+ , Cl^- , HCO_3^- , Ca^{2+} and PO_4^{3-} .

ROLE OF THE BONE MARROW IN HEMOPOIESIS

In early fetal life, ***hemopoiesis*** (= ***blood cell formation***) takes place outside the bone marrow, mainly in the ***liver and spleen*** (= ***extra-medullary hemopoiesis***). On the other hand, after birth all red blood cells and platelets and most white blood cells are formed in the bone marrow (= ***medullary hemopoiesis***).

In children, the marrow in all bones is active and is called ***red marrow***. However, this decreases gradually, and at the age of 20 years, the red marrow becomes confined to the flat bones (e.g. sternum, ribs and vertebrae) and upper parts of long bones (e.g. the humerus and femur), while the shafts of long bones will contain inactive ***fatty or yellow marrow***.

****** Although the red cell count in the blood is 500 times more than the white cell count, about **75 % of the marrow cells are leukocyte-producing cells** and only 25 % are maturing red cells. This is because the ***life span of the white cells is much shorter than that of the red cells***.

PROPERTIES OF THE BLOOD

- (1) The *blood colour is red* due to hemoglobin.
- (2) The *pH of arterial blood is 7.4* (that of venous blood is 7.36).
- (3) *Blood is opaque* due to its cellular elements.
- (4) The *blood specific gravity is about 1060* (that of the cells is about 1090 while that of the plasma is 1025-1030).
- (5) The *blood viscosity is 5 times that of water* due to its cellular elements and the plasma proteins (the plasma viscosity is 2 times that of water).
- (6) The *osmotic pressure (O.P.) of the plasma is about 5500 mmHg*. It is mostly due to *crystalloids* (electrolytes, glucose, urea, etc.), since that of the *plasma proteins is only about 25 mmHg* (which is called the *plasma colloid osmotic pressure or oncotic pressure*). The total plasma osmolality is *290-300 mOsm/litre*, and the plasma proteins contribute by only 0.5% (i.e. *less than 2 mOsm/litre*).

****** Solutions that have the same osmolality as that of the plasma are called *isoosmotic or isotonic solutions* e.g. a *0.9 % NaCl solution* (which is the same concentration of inorganic substances in the plasma, page 7).

On the other hand, solutions that have higher osmolalities than the plasma are *hyperosmotic or hypertonic* while those having lower osmolalities are *hypoosmotic or hypotonic*. A *5% glucose solution is initially isotonic*, but after glucose metabolism in the body the effects of this solution will be similar to those of hypotonic solutions.

GENERAL FUNCTIONS OF THE BLOOD

The blood *links the various body systems together* and greatly helps in producing *homeostasis* (= maintenance of the internal environment of the body constant as regards water, pH, electrolyte concentration, temperature, etc.). This is carried out through performing the following functions :

- (1) It is the *major transport medium in the body*. This is essential for
 - a- *Nutrition* (by transporting food from the GIT to the tissues).
 - b- *Respiration* (by transporting O_2 from the lungs to the tissues and CO_2 in the opposite direction).
 - c- *Excretion* (by transporting the waste products from the tissues to the excretory organs e.g. urea and creatinine to the kidneys).
 - d- *Regulation of metabolism* (by transporting hormones from various endocrine glands to the tissues and also by regulating their secretion),
 - e- *Regulation of the body temperature* by transporting heat to the skin (thus helping heat loss), and also by informing the hypothalamic thermoreceptors about the body core temperature (refer to metabolism).

- (2) It plays an essential role in *maintaining the acid-base balance* and keeping the pH of the body fluids constant (by its buffer systems).

(3) It is important in *the regulation of water balance*, specially in cases of hydration (by removing excess fluids via the kidneys).

(4) The blood viscosity produces *peripheral resistance* to the blood flow, which is essential for *maintenance of the arterial blood pressure*.

(5) The property of *blood clotting* is a major *hemostatic mechanism* for prevention of excessive blood loss in cases of hemorrhage.

(6) The *white blood cells provide the main defence mechanisms of the body* against a wide variety of microorganisms (refer to chapter 7).

THE HEMATOCRIT (H)

This is the percentage of the blood volume that is made up of cells, so it is also called the *packed cell volume (PCV)*. It is calculated as follows :

$$(H) = \frac{\text{blood cell volume}}{\text{total blood volume}} \times 100$$

Determination of (H)

A blood sample from the subject is placed in a special tube called *Wintrobe tube* that contains an anticoagulant and is then centrifuged.

The *red cells will be packed in the bottom of the tube* while the clear plasma remains above and the *white cells form a small buffy layer* just above the red cell column (figure 2). (H) is then calculated by dividing the blood cell column by the total blood column and multiplying by 100.

Normally, (H) averages *45 % in adults* (40- 47 % in males and 36-42 % in females) and *60 % in newly born infants* (due to polycythemia).

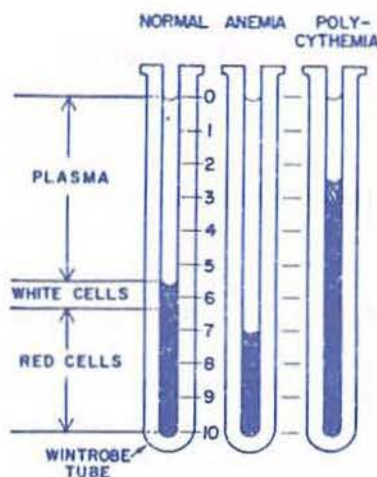


Figure 2 : The (H) in normal persons, and in anemia and polycythemia.

Factors that affect (H)

(H) is affected by *changes in the red cell volume relative to the plasma volume*. Accordingly, it is changed as follows :

- (1) It is *increased* in polycythemia (figure 2) and cases of hemoconcentration (e.g. when the plasma volume is decreased due to dehydration).
- (2) It is *decreased* in anemia (figure 2) and cases of hemodilution (e.g. when the plasma volume is increased due to hydration).
- (3) Its value in small blood vessels is less than its value in large vessels due to *plasma skimming* (refer to circulation) and is also greater in venous blood than in arterial blood due to *chloride shift* (refer to respiration).

Uses of (H)

The determination of (H) is used in (a) Estimation of the *blood volume* (see below) (b) *Diagnosis of anemia and calculation of some blood indices* (chapter 5) (c) Measurement of the *renal blood flow* (refer to kidney).

THE BLOOD VOLUME

NORMAL VALUES OF THE BLOOD VOLUME

The blood volume is normally about *8 % of the body weight, 80 ml/Kg of body weight and 3.2 litres / m² of body surface area*. Therefore, the total blood volume is about *5.6 litres in young adult males weighing 70 Kg*. These values are *less in females* (and also in obese persons) due to their greater body fat content (which is relatively avascular).

VARIATIONS OF THE BLOOD VOLUME

The blood volume may increase either physiologically (e.g. in pregnancy, high altitudes and following muscular exercise), or pathologically (e.g. in polycythemia vera). On other hand, its decrease is almost always pathological (e.g. in dehydration and after acute hemorrhage).

MEASUREMENT OF THE BLOOD VOLUME

The blood volume approximately equals the *sum of the plasma volume and red cell volume* (since the volume of other blood cells is negligible). These can be measured by the *dilution principle* (page 3) but it is unnecessary to measure both. *The blood volume can be estimated if only one of them is measured and the hematocrit (H) is known* (see below).

(A) MEASUREMENT OF THE PLASMA VOLUME

The plasma volume can be measured by 2 methods :

(1) **DYE METHOD** : A known amount of a dye e.g. *Evans blue* is i.v. injected into the subject, and after 5-10 minutes (which allows uniform dispersion of the dye in the plasma), the concentration of the dye in the plasma is determined. From the latter, the plasma volume can be determined, and the total blood volume can also be calculated if (H) is known as shown in the following example :

If the amount of the injected dye was 30 mg, and its plasma concentration after mixing was 0.01 mg/ml, then the *volume of distribution (i.e. the plasma volume)* = $30/0.01 = 3000 \text{ ml}$. If (H) was 40 %, this means that the determined plasma volume constitutes $100-H$ i.e. 60 % of the total blood volume. Accordingly, the total blood volume = $\text{plasma volume} \times 100/(100-H) = 3000 \times 100/60 = 5000 \text{ ml}$.

Characteristics of Evans blue

- (a) It is *non- toxic and is not metabolized in the body*.
- (b) It combines with the plasma proteins, so it does not rapidly escape into the tissue spaces (and is also *not rapidly excreted by the kidneys*).
- (c) It is *not adsorbed to the red cells* and does not enter or hemolyze them.
- (d) Its plasma level is easily measured by the spectrophotometer

(2) **ISOTOPE METHOD** : This is *more accurate than the dye method*. A sample of serum albumin is incubated with ^{131}I (= *radioactive iodine*) *in vitro*. An amount of this labeled albumin with *known radioactivity* is i.v. injected into the subject, and after 10-15 minutes, a blood sample is withdrawn, and the plasma radioactivity is measured. From the degree of dilution, the plasma volume can be determined , and the total blood volume can also be calculated if the (H) is known as described above.

(B) MEASUREMENT OF THE RED CELL VOLUME

This can be achieved *only isotopically*. A blood sample *from the subject* is incubated *in vitro* with an isotope that can bind to (or enter) the red cells e.g. *radioactive chromium* (^{51}Cr), *iron* (^{59}Fe) or *phosphorus* (^{32}P). The tagged (i.e. radioactive) red cells are then separated (by centrifugation), their radioactivity is measured and re-injected i.v. into the subject (in isotonic saline). After 10-15 minutes, a blood sample is withdrawn and the red cells are separated and their radioactivity is measured. From the degree of dilution of the tagged red cells , the patient's red cell volume(mass) can be determined, and the total blood volume can also be calculated if the (H) is known as shown in the following example :

Suppose the red cell volume was 2000 ml and (H) was 40 %. Since (H) is almost entirely determined by the red cell volume (page 9), then the determined red cell volume constitutes 40 % of the total blood volume. Accordingly, the total blood volume = *red cell volume* $\times 100 / (H) = 2000 \times 100 / 40 = 5000$ ml.

REGULATION OF THE BLOOD VOLUME

(A) REGULATION OF THE BLOOD CELL VOLUME

The regulation of the *red blood cell volume* is particularly important in maintaining the total blood volume constant because of their much greater number than other blood cells. This is normally achieved by the nearly *equal rates of their formation and destruction*. In cases of sudden blood loss e.g. due to *severe hemorrhage*, their volume is restored by 2 mechanisms :

(1) **A rapid compensatory mechanism** : Red cells are rapidly shifted from the blood stores (e.g. the spleen) into the general circulation.

(2) **A slow compensatory mechanism** : The rate of erythropoiesis in the bone marrow is increased as a result of O₂ lack (refer to chapter 5).

(B) REGULATION OF THE PLASMA VOLUME

Normally, the plasma volume is kept constant by 2 mechanisms :

(1) **Water balance** : This depends on the balance between water gain and water loss (page 3). Although it is a *slow mechanism*, it keeps the plasma volume constant over long periods.

(2) **Fluid exchange between the plasma & tissue(interstitial) fluid** : This is a *rapid but limited mechanism*. It occurs in the *capillaries* where the hydrostatic blood pressure acts as a *filtering force* and the oncotic pressure (or osmotic pressure) of the plasma proteins as a *reabsorbing force* (figure 3). The hydrostatic pressure is 30-40 (average 32) mmHg at the *arterial ends* of the capillaries, and 10-15 (average 12) mmHg at their *venous ends*, while the oncotic pressure averages 25 mmHg *allover*.

Accordingly, fluids are *filtered at the arterial ends* (where the hydrostatic pressure exceeds the oncotic pressure) and *reabsorbed at the venous ends* (where the oncotic pressure exceeds the hydrostatic pressure). Normally, the rate of *fluid filtration slightly exceeds the rate of fluid reabsorption*, and the excess fluid is drained back to the bloodstream by the *lymphatics (lymph vessels)* (figure 3).

If the plasma volume increases (e.g. in cases of hydration), the excess fluid is filtered in the tissue spaces due to rise of the hydrostatic pressure and decrease of the oncotic pressure. On the other hand, if the plasma volume decreases (e.g. in cases of dehydration), fluids are withdrawn from

the tissue spaces into the bloodstream due to rise of the oncotic pressure and decrease of the hydrostatic pressure. Thus, the plasma volume is kept constant in both conditions.

The *ICF is similarly affected as the tissue fluid* i.e it increases in hydration and decreases in dehydration. However, if such changes in the ICF are excessive, they would lead to damage of the cells (and this is the *cause why this mechanism is limited*).

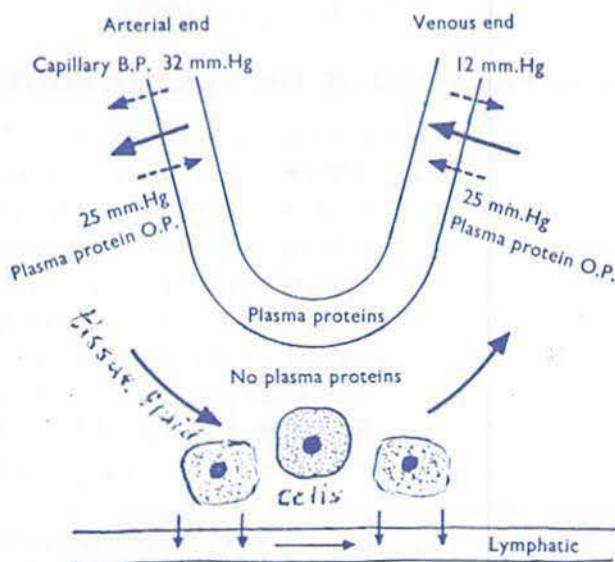


Figure 3 : Forces involved in the formation and drainage of the tissue (or interstitial) fluid.

LYMPH

This is *excess tissue fluid* that is drained by the lymph vessels. The latter unite forming the *thoracic and right lymph ducts* that drain into the *big veins at the base of neck*. Its composition is similar to that of the plasma but its protein content is generally lower. In the GIT, fats are absorbed in the lymph vessels (giving lymph a *milky appearance*). The lymphocytes that are formed in the lymphoid tissues *enter the blood mainly via the lymphatics* (chapter7).

CHAPTER 3

THE PLASMA PROTEINS

The plasma proteins include mainly *albumin, globulins and fibrinogen*, in addition to small amounts of other proteins (e.g. certain hormones, prothrombin and most of the other clotting factors). Their average amount is **7 gm % (6-8 gm %)** divided as follows :

(1) **Albumin** : This is the *most abundant plasma protein*. Its amount averages **4 gm % (3.5-5 gm %)**, and its molecular weight (m.w.) is **69000**.

(2) **Globulins (alpha 1 & 2, beta 1 & 2 and gamma globulins)** : These average **2.7 gm % (2.3 - 3.5 gm %)**, and their (m.w.) range from **90000 to 156000**.

(3) **Fibrinogen** : This is the *least abundant plasma protein*. Its amount averages **0.3 gm %**, and its (m.w.) is **340000**.

METHODS OF SEPARATION OF THE PLASMA PROTEINS

(1) **Plasma ultracentrifugation** : This technique leads to sedimentation of the various plasma proteins at different rates (so they can be separated).

(2) **Chemical separation** : This method is based on precipitation of the various plasma proteins, and it can be carried out by 2 methods :

a- **Precipitation by salts** : The plasma proteins can be precipitated by addition of different concentrations of certain salts. For example, albumin can be precipitated by full saturation of the plasma with *ammonium sulphate* while half saturation with this salt precipitates globulins.

b- **Fractional precipitation** : This is precipitation of the various plasma proteins at a low temperature by varying the plasma pH and addition of certain salts and alcohol.

(3) **Electric separation (electrophoresis)** : This is separation of the plasma proteins by passing a *constant electric current in the plasma*. It is the *most accurate method*, and is based on the following principles :

a- Proteins are *amphoteric substances* (i.e. they can ionize as acids or as bases) since they contain both carboxyl (COOH).and amino(NH₂) groups.

b- Each protein is neutral at a specific pH called its *isoelectric point (IEP)*, but it ionizes as a base in solutions that are acid relative to its IEP, and ionizes as an acid in solutions that are alkaline relative to its IEP.

The IEPs of the various plasma proteins are around pH 5, and since the blood pH (7.4) is alkaline relative to these IEPs, the plasma proteins ionize as acids (called proteinic acids). These acids dissociate into H⁺ and free proteinate⁻ anions (which combine with Na⁺ forming *Na proteinate*).

Accordingly, on passing an electric current in the plasma, the proteins migrate *towards the anode* by varying rates (depending on their molecular weights), where they can be separated on a paper strip (so this technique is called *paper electrophoresis*).

SITE OF SYNTHESIS OF THE PLASMA PROTEINS

All plasma proteins are synthesized *in the liver*, except gamma globulin which is synthesized *in lymphoid tissues* by the plasma cells (chapter 7).

THE ALBUMIN / GLOBULIN RATIO (A/G)

Normally, this ratio is *1.2 when electrophoresis is used* for separation of these proteins, and *1.6 - 1.7 when separated chemically* (see above). The determination of the A/G ratio is *important clinically* since it is altered by disease as shown in the following examples :

(1) It is *decreased* in (a) Advanced liver disease (due to decreased synthesis of albumin), so it is frequently used as *a liver function test*.

(b) Severe infections (due to increased synthesis of gamma globulin).

(2) It is *increased* when the globulin fraction is decreased e.g. in congenital agammaglobulinemia.

ORIGIN (SOURCE) OF THE PLASMA PROTEINS

This can be investigated in animals by a procedure called *plasma-pheresis* which is carried out as follows : A small amount of blood is withdrawn from the animal, anticoagulated and centrifuged. The *plasma is discarded* while the cells are reinjected into the animal in an isotonic saline solution. This process is repeated till the protein reserves in the body are exhausted and the plasma protein level, drops to 4 gm %. The animal is then given different types of food, and the rate of synthesis of different plasma proteins and their blood levels are measured. The results of this procedure showed that plasma proteins are primarily formed from *food proteins*, but they can be formed from *tissue proteins* if the protein content in food was low. .

(A) **Food proteins** : These are the most effective for synthesis of plasma proteins when (1) Their structure is similar to that of the plasma proteins (2) They are of a high biological value (i.e. rich in the essential amino acids). Certain proteins favour albumin formation (e.g. those from muscle) while others favour globulin formation (e.g. plant proteins).

(B) **Tissue proteins** : These are either *fixed tissue proteins* that cannot be converted into plasma proteins, or *reserve tissue proteins*. The latter can be converted into plasma proteins, and are 2 types :

(a) **Dispensable reserve proteins** : These are mobilized from the tissues to the liver during starvation where they are used for energy production and plasma protein formation.

(b) **Labile reserve proteins** : These are structurally similar to the plasma proteins. They are mobilized from the tissues to the bloodstream directly if the amount of the plasma proteins is suddenly decreased e.g. in case of hemorrhage (in the latter case, fibrinogen, globulins, then albumin are then gradually regenerated in that order).

FUNCTIONS OF THE PLASMA PROTEINS

(1) **Hemostatic function** : *Fibrinogen* is essential for blood coagulation.

(2) They share in production of **blood viscosity**, specially *fibrinogen and globulins* (due to their large m.w.) which causes *peripheral resistance* to blood flow. This helps *maintenance of the arterial blood pressure*, particularly the *diastolic pressure* (refer to circulation).

(3) Their **osmotic pressure** (about 25 mmHg) is essential for reabsorption of fluids from the tissue spaces at the capillary venous ends (figure 3), which *maintains the blood volume*. 70-80 % of the osmotic pressure is produced by *albumin* (because of its greater amount and smaller m.w.).

(4) **Buffer action** : They provide about 15 % of the *buffering power of the blood* e.g. a strong acid such as lactic acid can be buffered as follows :
 $\text{lactic acid} + \text{Na proteinate} \rightarrow \text{Na lactate} + \text{proteinic acid (a weak acid)}$.

(5) **Defence (immunity)** : Gamma globulins are *antibodies* that attack bacteria, so they are called *immunoglobulins* (see chapter 7).

(6) **Conservation function** : The plasma proteins combine loosely with many substances such as hormones (e.g. thyroxine and cortisol) and minerals (e.g. iron and copper). This serves as *a reservoir* for these substances and also *prevents their rapid loss in the urine*.

(7) **Control of capillary permeability** : The plasma proteins close the pores in the capillary walls, thus limiting their permeability. This favours development of edema in cases of hypoproteinemia (see below).

(8) **Carriage of CO₂** : CO₂ combines with the amino groups of the plasma proteins and is carried as *a carbamino compound (NHCOOH)*.

(9) **Nutritional function** : The plasma proteins can be utilized for nutrition of the tissues in cases of *prolonged starvation*.

(10) **Specific functions** : Certain plasma proteins exert specific functions e.g. the various hormones, the clotting factors and angiotensinogen.

****** Globulins and fibrinogen *increase the erythrocyte sedimentation rate* by favouring formation of rouleaux shapes of R.B.C.s (see chapter 5).

The following table shows the main functions of various plasma proteins

PLASMA PROTEIN	MAIN FUNCTIONS
<i>Albumin</i>	conservation function and osmotic pressure
<i>Globulins</i>	conservation function and defence (immunity)
<i>Fibrinogen</i>	blood coagulation and blood viscosity

HYPOPROTEINEMIA

This occurs due to either (1) Severe liver disease (due to deficient synthesis of the plasma proteins) (2) Nephrosis (due to loss of the plasma proteins in the urine) (3) Malnutrition (due to lack of proteins in the diet). Its main symptom is *generalized edema all over the body* (due to reduction of the osmotic pressure of the plasma proteins).

CHAPTER 4

HEMOSTASIS AND BLOOD COAGULATION

Hemostasis means *stoppage of bleeding and prevention of blood loss* when a blood vessel is injured. It occurs by **4 mechanisms** :

(A) Vasoconstriction (V.C.) of the injured vessel

This occurs as a result of the following :

- 1- Nervous reflexes (which are initiated by the pain of injury).
- 2- Myogenic contraction of the injured vessel (as a direct effect of trauma)
- 3- Release of serotonin and thromboxane A_2 from platelets (both are V.C).

The greater the trauma, the stronger the V.C. and vice versa, so *bleeding is severe in sharply-cut vessels* (due to weak V.C. as a result of minor trauma). V.C. is also weaker if the cut was longitudinal or irregular.

(B) Formation of a platelet plug (= temporary hemostatic plug)

The membranes of the platelets contain *receptors for collagen and ADP* (the latter has 3 types of receptors) as well as for *fibrinogen, thrombopoietin and the Von Willebrand factor* (a factor that is produced by the endothelial cells in the blood vessel walls and also by the platelets). The platelet plug is formed as follows :

1- As a result of injury of the blood vessel wall, the subendothelial collagen fibres are exposed.

2- The platelets adhere to collagen as well as to the *Von Willebrand factor* in the blood vessel wall via receptors on the platelet membrane (= *platelet adhesion or binding*).

3- Such binding of platelets initiates *platelet activation*. The activated platelets swell and develop pseudopodia, become sticky and release the contents of their granules, which contain ADP.

4- The released *ADP* binds to the nearby platelets (at the ADP receptors in their membranes) leading to their activation.

5- The newly activated platelets stick to the originally-activated platelets (= *platelet aggregation*) and also release ADP which, in turn, activates more and more platelets. Thus a *vicious circle of platelet activation* is elicited leading to formation of a *loose platelet plug* (which can usually stop bleeding, but only from small wounds).

In addition to ADP, platelet aggregation is also promoted by (1) a *platelet activation factor* (= *PAF*) which is secreted by the platelets and by the neutrophils and monocytes (2) *Thrombin* (3) *Thromboxane A_2* .

(C) Formation of a blood clot (= definitive hemostatic plug)

The platelet plug becomes firm when *fibrin threads* are deposited (see below), and this is essential for stoppage of bleeding from large vessels.

Clot retraction (= syneresis) : A few minutes after formation of the blood clot, it starts to shrink and within 20-60 minutes, it becomes about

one half its original size, and a clear **non-coagulable yellow fluid** called **serum** is squeezed out. As the clot retracts, it *pulls the edges of the injured vessel together, which helps hemostasis*.

Clot retraction was believed to be produced by a substance secreted by the platelets called **retractozyme**. However, it was found that the **platelets themselves contract** when they attach to the fibrin threads (leading to clot retraction) due to activation of their contractile proteins (**actin, myosin and thrombosthenine**) by **thrombin and Ca^{2+}** .

(D) Repair of the damaged blood vessel

Fibroblasts plus the vascular endothelial and smooth muscle cells grow and proliferate by the effect of a growth factor secreted by the **platelets, macrophages and endothelial cells** (= **platelet-derived growth factor, PDGF**). This cellular growth invades the blood clot till it is organized into **fibrous tissue** within 1-2 weeks, which repairs the injured vessel.

THE PLATELETS (THROMBOCYTES)

These are small granulated oval or round bodies 2-4 microns in diameter. They have **no nuclei** and are formed as detached bits from giant cells in the bone marrow called **megakaryocytes**. Their normal count averages **300000 per mm^3** , and their half life averages **4 days**. Their production is regulated by (1) The **colony-stimulating factors** that control the production of megakaryocytes (2) **Thrombopoietin**. This factor is produced by the liver and kidneys and it facilitates maturation of the megakaryocytes (and there are also thrombopoietin receptors on the platelets). Normally, about 60-75 % of the platelets are present in the circulating blood while the remainder is present mostly **in the spleen**.

STRUCTURE OF THE BLOOD PLATELETS

(A) **The platelet membrane** : This is extensively invaginated with a complex canalicular system in contact with the ECF. It contains **phospholipids** that include **platelet factor 3** (which plays an important role in blood clotting). There is also a coat of **glycoprotein** on its surface, which favours its **adhesion to injured endothelium** (but not to normal endothelium).

(B) **The platelet cytoplasm** : This contains the following :

(1) Glycogen, lysosomes, mitochondria, residuals of endoplasmic reticulum and Golgi apparatus (which synthesize enzymes and store Ca^{2+}).

(2) **Contractile proteins** (actin, myosin and thrombosthenine).

(3) **Dense granules** that contain **nonprotein substances** (specially ADP and serotonin).

(4) **Alpha granules** that contain **protein substances** (PDGF, clotting factor XIII, Von Willebrand factor and PAF).

FUNCTIONS OF THE PLATELETS

The platelets are primarily concerned with *hemostasis* through performing the following functions :

- (1) Formation of the *primary hemostatic plug* (see above).
- (2) Release of V.C. substances specially *serotonin and thromboxane A₂*.
- (3) Release of *platelet factor 3*, which is essential for blood clotting.
- (4) Induction of *clot retraction* by its contractile proteins (see above).
- (5) Stabilization of the blood clot by *factor XIII*.
- (6) Release of the *PDGF* which repairs of the damaged vessel wall.
- (7) Helping conversion of prothrombin to thrombin in sites of clotting because much of the prothrombin first attaches to the platelets that are already bound to the damaged tissues (Guyton, 2006).

THROMBOXANE A₂

This is a *prostaglandin-related substance* that causes *V.C. and promotes platelet aggregation*. It is formed from *arachidonic acid* as follows : This acid is converted to prostaglandin H₂ (PGH₂) by action of the cyclooxygenase enzyme. PGH₂ is then converted into thromboxane A₂ by action of the thromboxane synthetase enzyme.

****** In the vessel walls, PGH₂ is also formed from arachidonic acid, but here, it is converted into PG I₂ (*prostacyclin*) by action of the prostacyclin synthetase enzyme. PG I₂ *inhibits platelet aggregation and produces V.D.* (opposite actions to those of thromboxane A₂).

****** The balance between the actions of thromboxane A₂ and prostacyclin normally favours localized platelet aggregation and clot formation. This prevents excessive extension of the blood clot, which *keeps the blood vessels open and maintains the blood flow*.

****** Aspirin blocks the cyclooxygenase enzyme, so the production of both thromboxane A₂ and prostacyclin is decreased. However, the endothelial cells can readily produce new cyclooxygenase while the platelets cannot. Accordingly, the formation of prostacyclin is much more rapidly restored than thromboxane A₂, thus platelet aggregation is inhibited and clot formation is reduced. For this reason, small doses of aspirin taken for prolonged periods can prevent intravascular clotting.

Differences between plasma and serum

The composition of the plasma and serum is almost identical except that *serum lacks fibrinogen* (so serum is called *defibrinated plasma*) and *most clotting factors specially factors V & VIII* (because they are consumed during the clotting process (so serum doesn't clot while plasma do). Serotonin is also more in the serum due to its release from the platelets.

BLOOD COAGULATION (CLOTTING)

FACTORS REQUIRED FOR BLOOD CLOTTING

The following table shows the various factors (in numbers and by names) that are involved in the process of blood clotting :

Factor I	<i>Fibrinogen</i>
Factor II	<i>Prothrombin</i>
Factor III (TPL)	<i>Thromboplastin or Tissue factor</i>
Factor IV	<i>Ca²⁺ (Calcium ions)</i>
Factor V	<i>Proaccelerin, labile factor</i>
Factor VII	<i>Proconvertin, stable factor</i>
Factor VIII	<i>Antihemophylic globulin(AHG) or factor</i>
Factor IX	<i>Christmas factor</i>
Factor X	<i>Stuart Prower factor</i>
Factor XI	<i>Plasma thromboplastin antecedent (PTA)</i>
Factor XII	<i>Hageman factor (glass factor)</i>
Factor XIII	<i>Fibrin-stabilizing factor (Laki-Lorand factor)</i>
High molecular weight kininogen	<i>HMWK or Fitzgerald factor</i>
Prekallikrein	<i>Fletcher factor</i>
Platelet factor 3 (PL)	<i>Platelet phospholipid</i>
Von Willebrand factor	<i>Factor VIII-related antigen</i>

THE CASCADE MECHANISM OF BLOOD CLOTTING

The process of blood clotting involves a *cascade* (= series) of *reactions* which proceed in **2 different systems** (or pathways).

(A) THE EXTRINSIC SYSTEM (PATHWAY)

This occurs when there is *trauma (damage) to the vascular wall and the surrounding tissues*. It is a *rapid process* that may start and terminate in as little as 15 seconds, and it proceeds as follows (figure 4) :

(1) The damaged tissues release *TPL (factor III)*. This is a protein phospholipid mixture that *activates factor VII*.

(2) Active factor VII (VIIa) in presence of TPL, PL and Ca²⁺ *activates factor X* (an alpha-globulin that is synthesized by the liver).

(3) Active factor X (Xa) acts as a *prothrombin activator* that converts prothrombin to thrombin (in presence of factor V, Ca²⁺ and PL).

(B) THE INTRINSIC SYSTEM (PATHWAY)

This occurs *in absence of tissue damage* both *in vitro* by exposing the blood to electronegatively charged wettable surfaces (e.g. glass) and *in vivo* e.g. in cases of *intravascular clotting* (= *thrombosis*) which occur when blood is exposed to damaged endothelial cells or subendothelial collagen fibres. It is a *slow process* that begins to develop in 1-2 minutes and requires 6 minutes or more to form a clot. It proceeds as follows (figure 4)

(1) Exposure of blood to substances such as glass or collagen fibres leads to 2 effects (a) *Activation of factor XII* (catalyzed by HMW kininogen and kallikrein) (b) *Platelet aggregation and release of PL*.

(2) Factor *XIIa activates factor XI* (catalyzed by HMW kininogen).

(3) Factor *XIa activates factor IX* (the latter is also activated by factor VIIa formed in the extrinsic system as shown in figure 4).

(4) Factor *IXa forms a complex with factor VIIIa* (see below), and in the presence of PL and Ca^{2+} , it *activates factor X*.

(5) The same occurs as in steps 3, 4 and 5 in the extrinsic pathway.

**** The Von Willebrand factor forms a complex with factor VIII**, and the latter is activated when *separated from this factor (and also by thrombin)*. Accordingly, Von Willebrand factor *regulates the activity and blood level of factor VIII* besides inducing *platelet adherence* (page 17).

**** All clotting factors are globulins that are synthesized by the liver except** (1) Factor III (tissue thromboplastin) (2) Factor IV (Ca^{2+}) (3) Von Willebrand factor (a protein synthesized by the platelets and vascular endothelium) (4) Factor XIII (which is synthesized by the platelets).

**** The extrinsic system occurs only in vivo** (because it needs a tissue factor) whereas the *intrinsic system occurs both in vivo and in vitro*.

**** In cases of tissue damage, blood clotting occurs by both systems starting by the extrinsic** (due to release of factor III) then it continues by the intrinsic pathway (due to exposure of collagen).

**** Intravascular clotting (= thrombosis)** as well as clotting in *test tubes occur by the intrinsic pathway*. Thrombosis may occur in (a) **The veins** (if the blood flow becomes sluggish e.g. as a result of prolonged immobilization after surgical operations and delivery) (b) **The arteries** (at the sites of damaged intima e.g. due to atherosclerosis) (c) **The heart** : (at damaged areas of the endocardium e.g. the *mural thrombi* that overlie areas of myocardial infarction).

The thrombi may occlude the blood vessels completely or incompletely. On the other hand, bits of various sizes from the thrombi sometimes detach and form *emboli* that travel in the bloodstream to distant sites where they occlude other vessels e.g. the *pulmonary vessels* (commonly due to detached thrombi from the leg veins) and the *cerebral vessels* (commonly due to detached mural thrombus in the left ventricle).

****** Once blood clotting starts, *a vicious circle that promotes more clotting is initiated and continues by a +ve feedback mechanism till bleeding stops*. This is produced mainly by **thrombin** which once formed it enhances the clotting process by (a) Helping more platelet aggregation and release of PL (b) Activating factors V, VIII and XIII (specially factor V) and accelerating the actions of factors IX, X, XI and XII (c) Acting on prothrombin by autoactivation, leading to more production of thrombin.

INTERACTION BETWEEN BOTH SYSTEMS OF BLOOD CLOTTING

Blood clotting occurs by *both systems starting by the extrinsic* because it is a much more rapid process. The extrinsic system also interacts and potentiates the intrinsic system through (1) *The thrombin fformed by the extrinsic system* activates some of the factors involved in the intrinsic system (specially factors V and VIII) and accelerates the actions of most of the others (see above) (2) *The activated factor VII (VIIa) shares in activation of factor IX* (figure 4).

ROLE OF Ca^{2+} IN BLOOD CLOTTING

Ca^{2+} is an *essential catalyst* in the process of blood clotting. *Except for the initial steps, it is required for all other steps of blood clotting by both the extrinsic and intrinsic systems* (figure 4). Its normal plasma level is *9-11 mg %*, and if removed from the blood, clotting wouldn't occur. However, *in vivo reduction of blood Ca^{2+} to levels that stop blood clotting is incompatible with life*. This is because clotting stops only when the blood Ca^{2+} level is severely decreased (to about 4 mg %), and such level cannot be reached clinically since death would occur before it is reached due to *tetany* (= severe muscle spasm) that occurs when the Ca^{2+} level drops just below 7 mg % (refer to endocrine glands).

IMPORTANCE OF VITAMIN K IN BLOOD CLOTTING

Vitamin K is one of *the fat-soluble vitamins, so it requires bile to be absorbed in the small intestine*. It is a necessary for *conversion of glutamic acid to gamma-carboxyglutamic acid*, and *6 of the proteins involved in blood clotting require this conversion* before they are released into the circulation. These include *clotting factors II, VII, XI and X as well as proteins C and S* (page 27). Therefore, lack of vitamin K causes bleeding (so it is called *antihemorrhagic vitamin*). This can occur as a result of either (a) *Failure of absorption e.g. due to deficient bile flow* (b) *Deficient intestinal bacteria* (which normally synthesize vitamin K) (c) *Severe lack of the vitamin in diet*.

CLASSIFICATION OF THE CLOTTING FACTORS

The clotting factors can be classified into the following 5 groups :

(1) **Prothrombin group** : This includes factors **II, VII, IX and X**, all of which are *synthesized in the liver and vitamin K-dependent*.

(2) **Fibrinogen group** : This includes factors **I, V, VIII and XIII**, all of which *require thrombin for activation*. They are all synthesized in the liver except factor XIII, and a part of factor VIII (Von Willebrand factor) which are synthesized in the platelets.

(3) **Contact group** : This includes factors **XI and XII** (synthesized *in the liver*).

(4) **Calcium ions (factor IV)**.

(5) **Thromboplastin (TPL, tissue factor or factor III)**.

FACTORS THAT AFFECT BLOOD CLOTTING

(A) Factors that accelerate (promote) clotting

1. **In vitro** (a) Warming of blood (b) Contact of blood to -vely charged or wettable surfaces e.g. glass (c) Addition of foreign bodies to the blood.

2. **In vivo** (a) Blood stagnation (b) Roughening or damage of the vascular endothelial lining (c) Injection of vitamin K or adrenaline (the latter stimulates synthesis of fibrinogen & prothrombin and increases factor V activity).

(B) Factors that slow (inhibit) clotting

1. **In vitro** (a) Cooling of blood to zero °C (b) Collection of blood in non-wettable vessels e.g. paraffin or silicone-coated test tubes (so platelet aggregation is reduced) (c) Blood defibrination (= removal of the fibrin threads by special methods) (d) Addition of heparin to the blood (e) decreasing the blood Ca^{2+} concentration to low levels (see below).

2. **In vivo** (a) Vitamin K deficiency (b) Small doses of aspirin (see above) (c) Administration of heparin or dicumarol (see below).

ANTICOAGULANTS

(A) Anticoagulants used in vitro

1. **Sodium oxalate** : This *precipitates* Ca^{2+} as insoluble Ca oxalate, so the blood Ca^{2+} level is decreased (which prevents clotting).

2. **Chelating agents e.g. Na citrate and EDTA (Ethylene-Diamine Tetra-Acetic acid)** : These substances *bind* Ca^{2+} , so the blood Ca^{2+} level is decreased. Citrate is the anticoagulant used in transfused blood

3. **Heparin** : This can be used *both in vitro and in vivo* (see below).

The following table shows the differences between heparin and dicumarol :

	HEPARIN	DICUMAROL
Origin	Animal origin	Plant origin
Method of administration	By injection (specially i.v.)	Orally
Onset of action	Rapid (within a few minutes)	Slow (after 1-2 days)
Duration of action	Short (a few hours)	Long (a few days)
Antidote	Protamine	Vitamin K
Site of action	In vivo and in vitro	In vivo only
Clearing effect	Present	Absent
Mechanism of action	Mainly facilitating the action of antithrombin III	Competitive inhibition of vitamin K in the liver

(B) Anticoagulants used in vivo

1- HEPARIN : This is a highly negatively-charged mixture of sulfated polysaccharides with a strong acid reaction. It is a *natural anti-coagulant* that is formed by the *mast cells and basophil leukocytes*.

Mechanism of action : Heparin exerts its anticoagulant effect *mainly by facilitating the action of antithrombin III* which is a protease secreted by the liver that *inhibits the active forms of factors IX, X, XI and XII* (but not thrombin itself).

Another function of heparin (the clearing effect) : Heparin acts as a *cofactor for the lipoprotein lipase enzyme* (which hydrolyzes excess lipids). Thus, heparin indirectly decreases the level of lipids in the blood and such effect is known as the *clearing effect of heparin*.

The **antidote of heparin is protamine** (a highly basic protein that forms an irreversible complex with heparin). It is used clinically to neutralize overdoses of heparin.

2. DICUMAROL : This substance is of a *plant origin*. It is a *coumarin derivative*, and its structure is *similar to that of vitamin K*.

Mechanism of action : Dicumarol and other coumarin derivatives (e.g *warfarin*) exert their anticoagulant effect through blocking the action of vitamin K in the liver by *competitive inhibition*, thus the *synthesis of factors II, VII, IX and X is inhibited* leading to prolongation of both the clotting and prothrombin times (page 28).. Therefore, the *antidote of dicumarol* is the administration of *large doses of vitamin K*.

Dicumarol is taken *orally* and it acts *in vivo only* (because its site of

action is the liver). Its effects appear *after a latent period of 1-2 days* (during which the clotting factors already present in the blood are consumed) but such effects last for *a few days* (in contrast, heparin acts both *in vivo and in vitro* and is taken *only by injection*, and its effects appear *within a few minutes* but they last for *only a few hours*). Cases of thrombosis are treated *first by heparin* (due to its rapid onset of action), then treatment is continued by oral administration of dicumarol.

MAINTENANCE OF BLOOD FLUIDITY (NATURAL ANTI-CLOTTING MECHANISMS)

Normally, blood clotting is prevented (so that the blood fluidity is maintained) by the following mechanisms :

(1) The *rapid blood flow rate and the smooth and intact endothelium* in the blood vessels. *These prevent* (a) Accumulation and concentration of the clotting factors in the blood (b) Platelet adherence and aggregation.

(2) The balance between the actions of *thromboxane A₂ and PGI₂* (page 19).

(3) Synthesis of the various clotting factors in *inactive forms*.

(4) The *low concentration* of the various clotting factors, and their rapid removal by the *reticuloendothelial system, specially in the liver*.

(5) Presence of *heparin and antithrombin III*.

(6) *Adsorption of thrombin to the fibrin threads* (which inhibits its effect, thus preventing excessive clotting).

(7) Activity of the *fibrinolytic system*, a highly specialized system that removes the small clots that are continuously formed in the body.

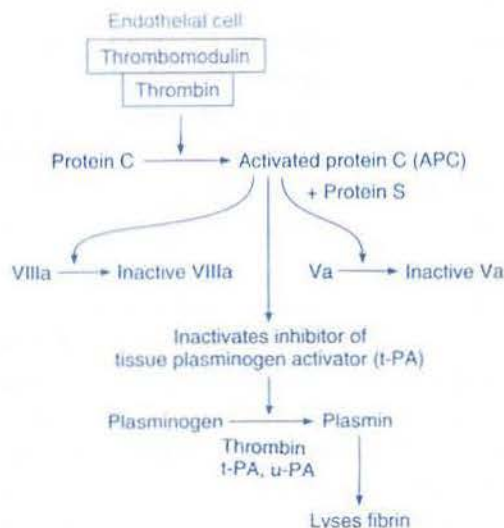


Figure 5 : The fibrinolytic system. Notice the roles of proteins C and S..

THE FIBRINOLYTIC (= PLASMINOGEN) SYSTEM

The liver produces an inactive globulin called *plasminogen* (= profibrinolysin) which can be converted to the active fibrinolytic enzyme called *plasmin* (= fibrinolysin) by *thrombin* and 2 types of activators (1) *urokinase Plasminogen Activator (u-PA)* (2) *tissue Plasminogen Activator (t-PA)*. The latter is released from injured tissues and vascular endothelium, and the liver produces an inhibitor to its action.

All vascular endothelial cells (except those in the cerebral micro-circulation) produce a protein called *thrombomodulin* (figure 5) that binds to thrombin, and such *thrombomodulin-thrombin complex* prevents blood clotting and causes lysis of fibrin as follows :

The complex *activates protein C*, and the activated protein C (APC) together with its co-factor (*protein S*) inactivate (a) Clotting factors V and VIII (which blocks the clotting reactions) (b) The inhibitor of t-PA (so increasing the formation of plasmin which helps lysis of fibrin).

Plasmin also lyses fibrinogen into fibrinogen degradation products (FDP) which inhibit thrombin. *Proteins C and S are produced by the liver and require vitamin K* (page 23).

FUNCTIONS OF THROMBIN

- (1) Conversion of fibrinogen into fibrin.
 - (2) Activation of clotting factors V, VIII and XIII and acceleration of the actions of factors IX, X, XI and XII (page 23).
 - (3) It induces platelet activation (page 17) as well as activation of the endothelial cells and leukocytes (Ganong, 2005).
 - (4) It helps clot retraction (page 18).
 - (5) It shares in activation of protein C (see above).
 - (6) It shares in conversion of plasminogen to plasmin (see above).
 - (7) It acts on prothrombin in a +ve feedback way to form more thrombin
- Thrombin is inhibited by* adsorption to fibrin and by the FDP (see above).

COMMON TESTS THAT EVALUATE HEMOSTATIC FUNCTION

(1) Clotting (coagulation) time

Determination of the clotting time tests the *coagulative ability of the blood*. It is measured from the time of blood withdrawal from the body till a firm clot is formed. It is determined by collecting a blood sample in a test tube, placing it in a water bath at 37 °C and every 30 seconds the tube is tilted. Clotting is indicated when blood does not drop from the tube and the time at which clotting occurs is measured using a stopwatch. *Normally, it is 3 – 8 (up to 10) minutes* and it depends on the *availability of the clotting factors* required for the *intrinsic system of clotting* (figure 4).

(2) Bleeding time

Determination of the bleeding time tests the efficiency of *hemostatic mechanisms other than blood clotting*. It is the time needed for bleeding to stop from an injured small blood vessel without formation of a blood clot. It is determined by pricking the tip of a finger (or the lobe of the ear), and the oozing blood is wiped away by a filter paper every 15 seconds without touching the skin. The time elapsing between the prick and stoppage of bleeding is recorded by a stopwatch. It depends on the *availability of the platelets*, and is *normally 1 - 3 (up to 4) minutes*.

(3) Prothrombin time

A blood sample is obtained and immediately oxalated or citrated to prevent clotting (so that no prothrombin is converted to thrombin). Blood is then centrifuged and the plasma is separated. To the plasma, Ca^{2+} and tissue thromboplastin (usually prepared from brain tissue) are added then it is incubated at 37°C and the time at which clotting occurs is recorded (normally *12-16 seconds*). It is a test for the *extrinsic system of blood clotting* (figure 4). Accordingly, it tests the quantities of *prothrombin and factor VII as well as factors I, V and X*.

****** The prothrombin time is commonly determined in patients having thrombotic tendencies to determine the required dose of dicumarol [which is usually adjusted to decrease the prothrombin group of clotting factors (page 24) to the extent that *doubles the prothrombin time*].

DISEASES CHARACTERIZED BY BLEEDING

Excessive bleeding may occur due to :

(A) Decreased platelet count (thrombocytopenia) : This occurs in a disease called *purpura* (see below).

(B) Deficient blood clotting : This occurs due to lack of any of the clotting factors, which may result from either :

(a) A severe liver disease (in which most clotting factors are deficient).

(b) Vitamin K deficiency (which leads to lack of factors II, VII, IX & X).

(c) A congenital abnormality : Any clotting factor (except factors II, III and IV) can be congenitally-deficient, leading to the following diseases that are characterized by bleeding :

1- *Hemophilia*, which is due to lack of factor VIII, IX or XI (see below).

2- *Parahemophilia* (which is due to lack of factor V).

3- *Afibrinogenemia* (which is due to lack of factor I).

4- *Hypoconvertinemia* (which is due to lack of factor VII).

5- *Hageman trait*, which is due to lack of factor XII (here blood clotting is slow in glass only but clinically there is little bleeding tendency).

6- *Von Willebrand disease* (which is due to lack of the V.W. factor).

7- *Congenital deficiency of factors X or XIII*.

PURPURA

This is a bleeding disease characterized by occurrence of *small punctate hemorrhages throughout all tissues*, specially in the skin where they appear as *purplish blotches* (hence the name). In most cases, it is due to severe reduction of the platelet count (*below 40000 - 50000/mm³*), so the condition is often called *thrombocytopenic purpura*. However, in some cases the platelet count is normal but the platelets themselves are abnormal, so these cases are called *thrombasthenic purpura*.

Lack of platelets causes spontaneous hemorrhages due to (a) Deficient formation of platelet plugs (b) Weak V.C. of the injured vessels (c) Poor clot retraction. Such effects lead to *prolongation of the bleeding time while both the clotting and prothrombin times are normal* (the clotting process occurs normally because it does not require a large number of platelets). Thrombocytopenia may be due to either *autoimmunity, hypersplenism or severe destruction of the bone marrow*.

****** The bleeding time is also prolonged in (a) *Scurvy* (vitamin C deficiency) due to weak vascular walls (b) *Deficiency of Von Willebrand factor* (due to defective platelet adhesion) (c) *Prolonged use of aspirin* (due to defective platelet aggregation).

Treatment of purpura

- (1) Fresh whole blood transfusion (an emergency treatment).
- (2) Splenectomy is usually helpful.

HEMOPHILIA

This is a bleeding disease caused by *congenital deficiency of certain clotting factors*. According to the deficient factor, it is classified into 3 types :

- (1) *Hemophilia A or classic hemophilia* (85 %) due to congenital deficiency of factor *VIII*.
- (2) *Hemophilia B* (15%) due to congenital deficiency of factor *IX*.
- (3) *Hemophilia C* (5%) due to congenital deficiency of factor *XI*.

Hemophilia is *inherited as a sex-linked characteristic* because the deficient factors are *genetically transmitted by way of the X sex chromosome* as a *recessive trait*. Males are affected if one of these factors is lacking due to defect of the responsible genes in their X chromosome, while females are affected only if such defect is present in both X chromosomes. For this reason, *females are much less affected, and most patients are males*. However, if the female had a defect of the responsible genes in one X chromosome only (while the other has normal genes), she will be a *hemophilic carrier*. In this case, if the X chromosome that has the genetic defect is transmitted to one of her sons, he will be hemophilic

while if it is transmitted to one of her daughters, she will only be a hemophilic carrier (provided her father is normal).

****** It is possible to have a hemophilic female if the father is hemophilic and the mother is a carrier, or if both parents are hemophilic (which is rare).

As a result of deficiency of the clotting factors, *the clotting time is markedly prolonged while both the bleeding and prothrombin times are normal*. This leads to marked bleeding after minor injuries, wounds or operations (e.g. tooth extraction).

Treatment of hemophilia

(1) Supplying the patient with the deficient clotting factor (this can be detected by a special test called the *thromboplastin generation test*).

(2) *Whole blood transfusion* at intervals and after severe bleeding.

The following table shows the differences between hemophilia & purpura

	HEMOPHILIA	PURPURA
Heredity	Inherited	Not inherited
Genetic abnormality	Abnormality of genes on the X chromosome	No genetic abnormality
Affected sex	Mostly males	males and females equally
Characteristics	Marked bleeding after minor injuries and operations	Spontaneous bleeding in all tissues specially the skin
Clotting time	Prolonged	Normal
Bleeding time	Normal	Prolonged
Cause	Lack of clotting factors VIII, IX or XI	Lack of platelets below 40000 - 50000/mm ³
Types	3 types (A, B and C)	2 types (see text)

****** *The prothrombin time is normal in both hemophilia and purpura.*

****** Although the clotting time is normal in purpura, and the bleeding time is normal in hemophilia, yet there is *a bleeding tendency in both conditions (indicating that all mechanisms of hemostasis are required for efficient stoppage of bleeding)*.

****** A group of specific proteins that are related to blood clotting and fibrinolysis were recently identified. More than 20 of these proteins have been described and they are now called **annexins**. *Annexin II* forms a platform on the endothelial cells on which the components of the fibrinolytic system interact, producing fibrinolysis. *Annexin V* forms a shield around the phospholipids involved in clotting and exerts an antithrombotic effect.

CHAPTER 5

THE RED BLOOD CORPUSCLES (R.B.C.s)

The R.B.C.s (red cells or *erythrocytes*) are non-nucleated circular biconcave discs (figure 6) that have *an average life span of about 120 days*. Their average diameter is 7.5 microns, thickness 2 microns and volume 87 cubic microns. Their normal count averages **5 million/mm³** (*5.4 million in adult males and 4.8 million in adult females*).

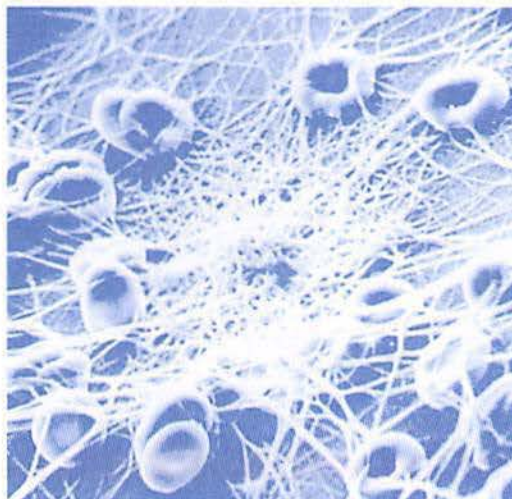


Figure 6 : Normal red blood corpuscles entangled in fibrin threads.

POLYCYTHEMIA (= Erythrosis or Erythremia)

This means increased R.B.C.s count, and it is 2 types :

(1) **Primary polycythemia (= polycythemia vera)** : This is a disease in which there is excessive production of R.B.C.s due to *an unknown cause*. Their count may reach 7-8 million/mm³ leading to *increased hematocrit, blood volume and viscosity*. The increased blood volume tends to increase the venous return while the increased viscosity tends to decrease it (so the cardiac output usually remains normal). On the other hand, the increased peripheral resistance (due to the increased blood viscosity) tends to elevate the arterial blood pressure. The condition also leads to *stagnant hypoxia* (due to the sluggish blood flow) which is associated with *cyanosis* (refer to respiration).

(2) **Secondary (= physiological) polycythemia** : This normally occurs whenever the body suffers *O₂ lack* (which stimulates red cell production in the bone marrow). The condition occurs in individuals living at *high altitudes* (in whom the red cell count may reach up to *6-8 million/mm³*) and also in *newly-born infants* (due to the relative O₂ lack during intrauterine life) in whom the hematocrit is about 60 %. In the latter cond-

ition, the excess red cells are gradually hemolyzed so that the hemoglobin concentration falls from about 20 to about 12 gm % within the first 3 months after birth. The iron released from the degraded hemoglobin (plus that stored in the body) provides a sufficient reserve for normal erythropoiesis up to the *first 4-6 months of life* (after which exogenous iron must be supplied, otherwise the infant would suffer iron deficiency anemia).

STRUCTURE OF THE RED BLOOD CELLS

The red cell is formed of a viscous solution (cytoplasm) enclosed by a cell membrane. The cytoplasm is formed mainly of *hemoglobin (Hb)*. Each red cell contains about *30 pg Hb* (pg = picogram = micromicrogram = 10^{-12} gram), so about *900 gram Hb are present in the blood*. It also contains electrolytes (specially K^+ and HCO_3^-) and several enzymes e.g. *carbonic anhydrase and glucose-6-phosphate dehydrogenase (G6PD)*.

The red cell membrane is formed of 3 layers (a) An outer layer formed of glycoprotein (b) A middle layer formed of phospholipids (c) An inner layer formed of mucopolysaccharide. The biconcave shape of the red cells is produced by 2 proteins in the their membranes called *ankyrin and spectrin* (their defects have been reported as causes of spherocytosis).

FUNCTIONS OF THE RED BLOOD CELLS

(1) The red cells play an important role in producing *blood viscosity* which is essential for maintenance of the diastolic arterial B.P.

(2) Hemoglobin is *essential for O_2 carriage*. It is also *essential for CO_2 transport*, and the *carbonic anhydrase enzyme* is important for this function. In addition, it is an *important buffer* (refer to respiration).

(3) The main function of the red cell membrane is to *keep Hb inside the red cells and prevent its escape into the plasma*. Its glycoprotein layer contains the *specific agglutinogens* that determine the blood group (refer to chapter 6). In addition, it allows *free diffusion of O_2 and CO_2* in both directions, and the biconcave shape of the red cells provides the *largest possible surface area* for this purpose (normally about 140 micron^2).

****** The red cell membrane is also permeable to various electrolytes. However, *K^+ is retained inside the red cells while Na^+ is pumped outside* (by energy derived from glycolysis), so normally the intracellular K^+ concentration is higher while that of Na^+ is lower than their plasma levels.

****** The red cell membranes are *not stretchable* but have a *high degree of flexibility* which allows the red cells to be *deformed into almost any shape*. Such *red cell deformability* is important because it enables these cells to (a) Compress and pass through the narrow channels in the vascular system e.g. the capillaries and the splenic sinusoids (b) Resume their normal shapes on leaving these channels.

HEMOGLOBIN (Hb)

This is a chromoprotein with a molecular weight 64450. Its molecule is globular in shape, and is made up of *4 subunits* (figure 7 left), each of which is formed of a *heme molecule conjugated to a polypeptide chain* (figure 7 right). The heme is formed of a *porphyrin derivative containing one ferrous (Fe^{2+}) atom*. Thus, each Hb molecule contains *4 Fe^{2+} atoms* and *2 pairs of polypeptide chains*, which are collectively called *globin*.

The Hb in normal adult human beings is called *HbA*, and its normal amount *averages 15 gm %* (about 16 gm % in males and 14 gm % in females). It contains *2 alpha polypeptide chains* (each containing 141 amino acids) and *2 beta polypeptide chains* (each containing 146 amino acids), so it is referred to as *$\alpha_2\beta_2$ Hb*.

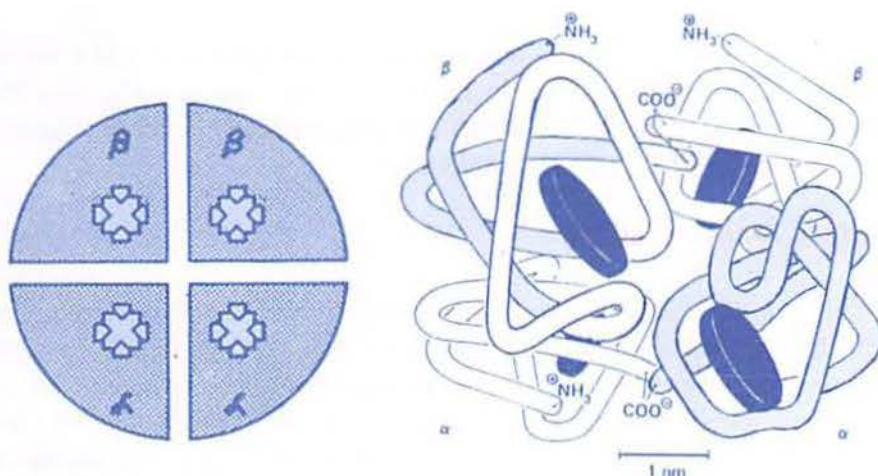


Figure 7 : 2 diagrams showing the structure of the Hb molecule (the black discs in the right diagram represent the heme molecules).

ABNORMALITIES OF HEMOGLOBIN PRODUCTION

These abnormalities are usually inherited and they are 2 types :

(1) The hemoglobinopathies : These are conditions in which abnormal Hbs (i.e. having different polypeptide chains from those of Hb A) are produced. Such abnormalities are due to mutations in the globin genes, and over 1000 of abnormal Hbs have been described in humans. Some of these Hbs are harmful e.g. Hb S (see below) while most of the others are harmless e.g. *Hb A₂*, *Hb C*, *Hb E*, *Hb I* and *Hb J*.

About **2.5 % of Hb in normal adult persons is Hb A₂** which contains **2 alpha and 2 delta poly-peptide chains ($\alpha_2\delta_2$ Hb)**.

****** Also normally, there are small amounts of *glycated Hb A* derivatives. One of them (**HbA_{1c}**) has a glucose molecule attached to each beta chain. The quantity of HbA_{1c} increases in poorly controlled diabetes mellitus and it decreases with insulin treatment, so its measurement is used as an index in evaluating the control of that disease.

(2) The thalassemias : These are conditions in which the Hb polypeptide chains are normal in structure but they are produced in decreased amounts (or some of them may be absent) because of defects in the regulatory portion of the globin genes.

There are 2 types of this abnormality, *alpha and beta* (according to the deficient polypeptide chain). The red cells are *hypochromic and also rapidly hemolyzed in the circulation* (due to an intrinsic defect) leading to a severe type of anemia called *mediterranean or Cooley's anemia*.

HEMOGLOBIN F

This is the Hb present in the red cells of *the fetus*, and it is normally replaced by HbA soon after birth. Its O₂ carrying capacity is greater than that of HbA, and it contains *2 alpha and 2 gamma polypeptide chains* (= *alpha₂ gamma₂ Hb*) (refer to respiration).

HEMOGLOBIN S (Hb S)

This is a type of Hb in which the alpha polypeptide chains are normal but in the *beta chains* one glutamic acid residue is replaced by a valine residue. At low O₂ tensions, Hb S polymerizes and this causes the red cells to become sickle shaped, hemolyze and form aggregates that block blood vessels. The result is a severe hemolytic anemia called *sickle cell anemia*. This anemia is *more common in black races*, and although it is dangerous (and may be fatal), it has a benefit in Africa because the sickle cell gene also *provides resistance to one type of malaria*.

O₂ - Hb COMBINATION

O₂ combines to Fe²⁺ in the heme molecule forming oxyHb. However, the iron *remains in the Fe²⁺ (ferrous) state*. Certain drugs and oxidizing agents convert Fe²⁺ in heme to the Fe³⁺ (ferric) state, producing a dark-coloured compound called *metHb* which is *unable to carry O₂* and causes *a dusky discolouration of the skin that resembles cyanosis*. Normally, some Hb is oxidized to metHb, but this is rapidly converted back again to Hb by activity of the *NADH metHb reductase enzyme system in the red cells*. Congenital absence of this system leads to excessive formation of metHb (= *hereditary methemoglobinemia*) which is a dangerous condition.

FATE (METABOLISM) OF Hb

Old red cells become fragile and are hemolyzed during passage at tight spots in the circulatory system and in the spleen. The released Hb is engulfed by cells of the *reticuloendothelial system* (refer to chapter 7) where it is *metabolized resulting in formation of bile pigments as end products*. This occurs as follows :

Hb is first broken down into heme and globin. Fe^{2+} atoms are separated from the heme molecules then they are stored as *ferritin* (which together with globin will be re-used for Hb synthesis). The remaining part of the heme molecule is a bile pigment called *biliverdin*. This is reduced to another pigment called *bilirubin* which is liberated into the bloodstream. On reaching the liver, bilirubin is conjugated to glucuronide and the resulting compound (*bilirubin glucuronide*) is excreted in bile. This compound is then converted by bacterial action in the large intestine into another pigment called *stercobilinogen*, some of which is excreted in the stool (giving it a *brown colour*) while the majority is reabsorbed back into the bloodstream (refer to digestion). Most of the latter is re-excreted by the liver in bile, while a small amount is excreted by the kidneys in the urine and is called *urobilinogen* (figure 8).

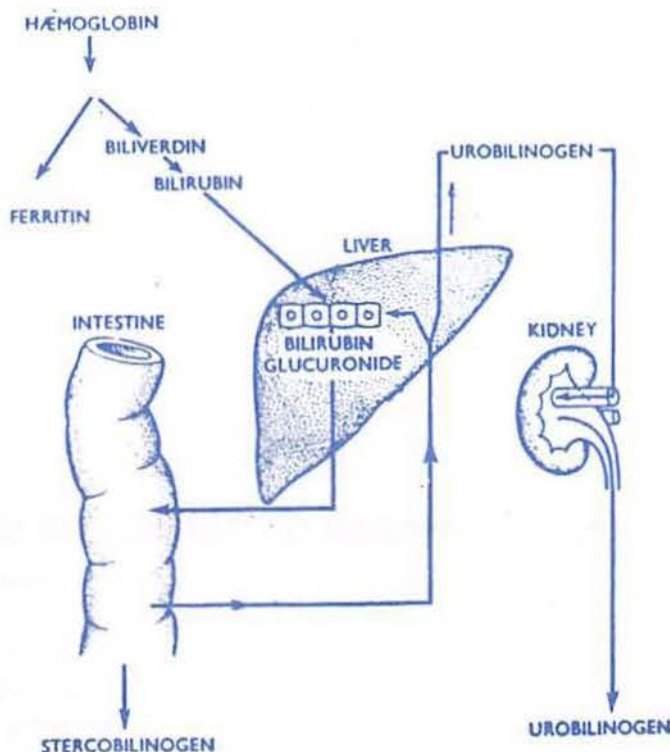


Figure 8 : Metabolism of hemoglobin and formation of the bile pigments.

ESTIMATION OF Hb CONTENT IN THE BLOOD

The blood Hb content can be measured by the *Sahli hemometer* or *hemoglobinometer* (figure 9). This method depends on matching the blood colour with a standard solution that has the colour of normal blood. *N/10 HCl solution* is placed in the dilution (test) tube till the mark 10, then 20 cmm blood are withdrawn from the subject (by a special pipette provided with the apparatus) and added to the HCl in the dilution tube. The red colour of the blood turns brown because HCl changes Hb into the brown coloured *acid hematin*. Distilled water is then added drop by drop to the sample till its colour *becomes the same as that of the standard*. The Hb content (in gms) and its % of normal can then be directly obtained from gradations on the dilution tube. *In polycythemia it may reach as high as 130 % while in anemia it is usually below 60 %.*

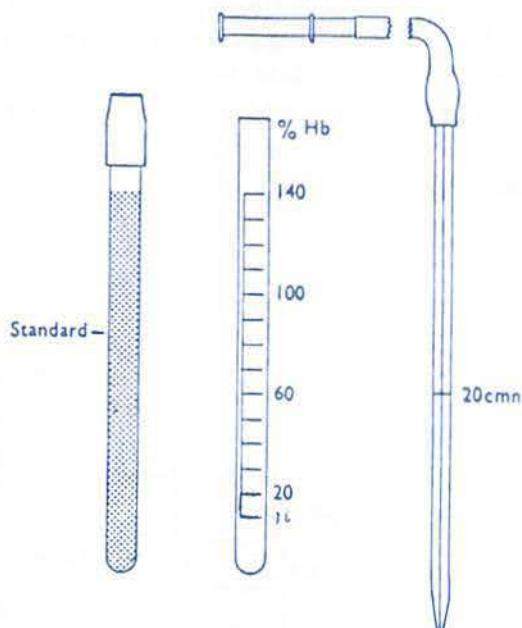


Figure 9 : Components of Sahli hemometer. The dilution (test) tube in the middle and the pipette used for withdrawal of blood on the right.

SITE OF FORMATION (ORIGIN) OF THE RED CELLS

During early fetal life, red cells are formed in the *area vasculosa of the yolk sac* then, till the end of the 6th month, they are formed in the *liver and spleen* (= *extramedullary origin*). During the last 3 months of pregnancy and after birth, they are formed in the *bone marrow* (= *medullary origin*). In children, all bones contain *active (red) marrow*. However, the red marrow gradually decreases with age, so that at the age of about 20 years, the active marrow becomes restricted to the upper ends

of long bones (specially the *femur and humerus*) and the *flat bones* (the ribs, sternum and vertebrae), while in the shafts of long bones, the red marrow becomes replaced by *inactive (yellow or fatty) marrow* (page 7). Also, with the advance of age, red cell production declines specially in the long bones (figure 10).

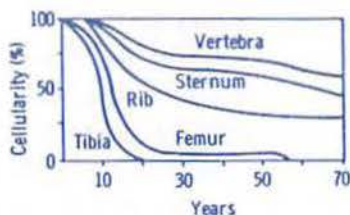


Figure 10 : Effect of age on red cell production in various bones.

PHYSIOLOGICAL FACTORS THAT AFFECT RED CELL COUNT

(1) **Age** : It is *high in newly-born infants* due to polycythemia (page 31). and low in old individuals.

(2) **Sex** : It is higher in males mainly because erythropoiesis is stimulated by *the male hormone* (= testosterone).

(3) **Exercise, hot weather and emotions** (in which the red cell count is increased due to *spleen contraction* induced by adrenaline secretion).

(4) **High altitudes** (the red cells increase due to polycythemia, page 31).

(5) **Diurnal variation** : About 5 % change in the red cell count occurs during 24 hours, being highest in the evening and lowest during sleep.

****** During pregnancy the increase in blood volume is mainly due to an increase in the plasma volume. This causes a relative decrease of the Hb content leading to what may be called *physiological anemia*.

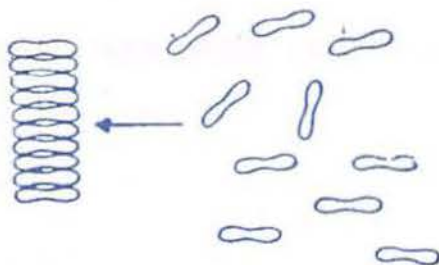


Figure 11 : Formation of rouleaux red cell shapes (on the left).

ERYTHROCYTE SEDIMENTATION RATE

This is the rate at which the red cells sediment (sink) when blood is placed vertically in a narrow tube. Red cells sediment because (a) Their density is greater than that of the plasma (b) They tend to aggregate to form *rouleaux shapes* (figure 11) Rouleaux formation is different from red cell agglutination (agglutinated red cells are irreversibly bound together and cannot be separated, chapter 6)..

ESR is determined by the *Westergren method* as follows : A blood sample from the subject is anticoagulated by addition of 3.8 % Na citrate solution at a ratio of *1 part citrate to 4 parts blood* (e.g. 0.5 ml citrate to 2 ml blood). Blood is then placed in a Westergren tube (length 30 cm and diameter 2.5 mm) which is fixed in a vertical position. The cells sediment (at first slowly then rapidly as they form rouleaux shapes) and after one hour, the height of the column of clear plasma (in mm) on top of the sinking red cells is measured, and this represents the ESR in mm / hour. A second measurement can be obtained after 2 hours and in this case, the ESR is calculated as follows :

$$\text{ESR} = (\text{first hour reading} + 1/2 \text{ second hour reading}) / 2$$

NORMAL VALUES OF THE ESR

In males, it is *4-6 mm / hour* while it is higher in females (*6-10 mm /hour*) because the red cell count in females is lower. It may be zero if the plasma is very viscous.

FACTORS THAT AFFECT THE ESR

(1) **Plasma proteins** : The ESR increases if the *albumin level is decreased* and when the *level of fibrinogen or globulins increases*. The latter effect is probably due to decrease of the normal negative charges of the red cells (which reduces their repulsion and helps rouleaux formation).

(2) **Red cell count** : The ESR is decreased in polycythemia and increases in anemia *except in iron deficiency* (probably due to reduction of the intrinsic ability of the red cells to sediment).

COMMON CAUSES THAT INCREASE THE ESR

(A) **Physiological causes** : The ESR is normally high in females during *menstruation* (about 15 mm /hour) and *pregnancy* (20 mm /hour or more) and in cases of *increased temperature* and after *muscular exercise*.

(B) **Pathological causes** : The ESR increases in cases of *inflammation and tissue destruction* due to increased fibrinogen or globulins Thus it increases in (1) Acute and chronic infections e.g. tonsillitis, appendicitis and tuberculosis (2) Severe trauma e.g. in fractures (3) Myocardial infarction (4) Degenerative and neoplastic diseases (5) Rheumatic arthritis.

CLINICAL IMPORTANCE OF ESR ESTIMATION

Because the ESR is changed in a great variety of conditions (see above), its alteration is *not specific (i.e. not diagnostic) for any particular disease* and gives no indication about the nature of the underlying disorder. However, *repeated ESR estimations help in prognosis* and follow up of the activity of the disease and its response to treatment.

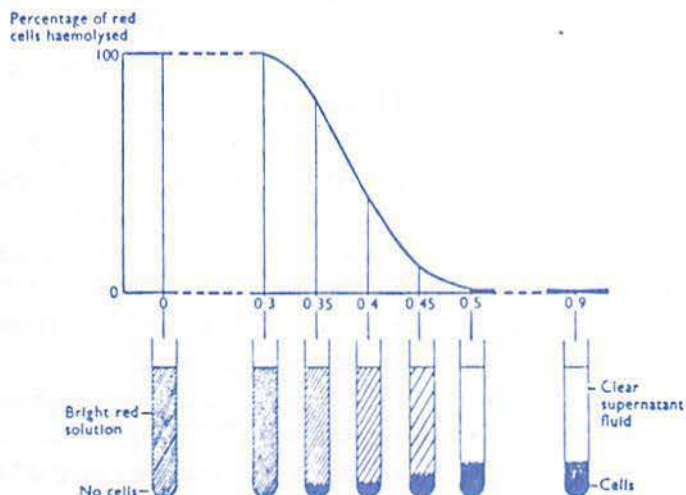


Figure 12 : Hemolysis in different concentrations of NaCl solutions.

OSMOTIC FRAGILITY OF THE RED BLOOD CELLS

A 0.9 % NaCl (saline) solution is isotonic with plasma. When red blood cells are suspended in hypertonic saline solutions, water diffuses out of the cells and they shrink (= *red cell crenation*). On the other hand, when they are suspended in hypotonic saline solutions, water diffuses into the cells so they swell and may rupture (= *hemolysis*).

The ability of the red cells to resist hemolysis in hypotonic saline solutions determines their osmotic fragility. The biconcave shape of the red cells allows 45-65 % increase in their volumes before they rupture. This range indicates that the *R.B.C.s vary in their fragility* (probably because the *older cells are more fragile and rupture before the young ones*) so that *hemolysis normally starts in about 0.5 % NaCl solution and is complete in about 0.35 % NaCl solution* (figure 12).

FACTORS THAT AFFECT RED CELL FRAGILITY

(1) Congenital and inherited defects of hemopoiesis

(a) *Sickle cell anemia and thalassemia* : These conditions are associated with increased fragility of the red blood cells which renders these cells to be readily hemolyzable (page 34).

(b) **Hereditary spherocytosis** : The red cells are spherical due to abnormalities of the protein network that maintains the shape and flexibility of their membranes, particularly *spectrin and ankyrin* (page 32)..

The spherocytes are smaller than normal cells and are more fragile, so they *start to hemolyze at about 0.7 % saline solution* and are completely hemolyzed at about *0.45 % saline solution*.

(2) **Acquired anemias** : In iron deficiency anemia, the red cells are flattened but they are not fragile, so they resist hemolysis more than normal. On the other hand, in macrocytic anemias, the abnormal red cells are fragile, so they are more readily hemolyzable than normal.

(3) **Ionic changes in the blood** : Normally, the red cells in the *venous blood become slightly spherocytic* (and accordingly *more fragile*) due to the chloride shift phenomenon (refer to respiration).

(4) **Red cell age** : In old red cells, the *power of Na^+ pump is decreased*. Accordingly, Na^+ accumulates inside these cells and the resulting rise of the osmotic pressure causes water diffusion into them, so they become fragile and hemolyze more easily than normal..

(5) **The G6PD enzyme** : This enzyme catalyzes glucose oxidation in the red cells. Such oxidation generates *NADPH* (Nicotinamide-Adenine Dinucleotide Phosphate) which is *needed for maintenance of the normal red cell fragility*. Accordingly, the red cells become fragile and hemolyze easily if the G6PD enzyme is congenitally deficient.

CAUSES OF HEMOLYSIS

- (1) Intravenous injection of excess amounts of *hypotonic fluids*.
- (2) *Incompatible (mismatched) blood transfusion* (refer to chapter 6).
- (3) Deficiency of the *G6PD enzyme* (see above).
- (4) *Snake venom*, some chemicals (e.g. arsenic) and bacterial toxins.
- (5) *Hereditary spherocytosis, abnormal Hbs (e.g. Hb S) and thalassemia*.
- (6) *Blood freezing* :The formed ice crystals cause disruption of the red cells and hemolysis during thawing of blood (i.e. liquefaction by heat).
- (7) *Fat solvents* e.g. ether (destroy the lipoprotein in cell membranes).
- (8) Immune reactions that cause formation of *red cell antibodies*.

EFFECTS AND DANGERS OF HEMOLYSIS

- (1) *Jaundice* (due to excessive formation of bilirubin).
- (2) *Increase of the plasma colloid osmotic pressure* (from 25 mmHg to about 70 mmHg), which prevents tissue fluid formation.
- (3) *Hemoglobinuria* (= Hb excretion in the urine). Hb is also precipitated in the renal tubules leading to *oliguria* and may be *renal failure*.
- (4) *Hb dilution* by the plasma. This *shifts the O_2 dissociation curve to the left* leading to *less O_2 delivery to the tissues* (refer to respiration).

- (5) *Tissue hypoxia* due to both Hb loss and Hb dilution.
- (6) *Acidosis* due to (a) Renal insufficiency (b) Decreased blood buffering power after loss of Hb (c) The decreased O₂ delivery to the tissues.
- (7) *Shock due to V.D.* , which occurs secondary to release of certain *V.D. substances* from the hemolyzed red cells specially *histamine*.
- (8) *Hyperkalemia* due to release of K⁺ from the hemolyzed red cells.

FACTORS THAT AFFECT ERYTHROPOIESIS (R.B.C.s FORMATION)

(1) BLOOD O₂ TENSION

Erythropoiesis is stimulated when the blood O₂ tension is decreased (e.g. in high altitudes and after hemorrhage) and vice versa. *O₂ lack (hypoxia) is the most powerful stimulus for erythropoiesis* through inducing secretion of the hormone erythropoietin from the kidneys (see next).

(2) THE KIDNEYS

The endothelial cells of the peritubular capillaries in the kidneys secrete a hormone called *erythropoietin* (a glycoprotein having a m.w. 34000) which stimulates red cell formation in the bone marrow through *increasing the number of the erythroid committed stem cells*.

The release of erythropoietin is stimulated by *O₂ lack, the male sex hormone (androgen) and cobalt salts*, and is facilitated by catecholamines and in conditions of *alkalosis* (e.g. at high altitudes). About 15 % of erythropoietin is secreted by the hepatocytes and Kupffer cells of *the liver*.

(3) HORMONES

Erythropoiesis is stimulated by several hormones specially *androgen* (see above) and *thyroxine*.

(4) THE LIVER

The liver forms the globin part of Hb, stores vitamin B₁₂ and several minerals required for red cell formation specially iron (see below), and also secretes some erythropoietin.

(5) THE BONE MARROW

A healthy bone marrow is essential for normal hemopoiesis (including erythropoiesis), so its damage (e.g. by radiation or disease) results in a severe type of anemia known as *aplastic anemia* (see below).

(6) DIET

For adequate erythropoiesis, the diet should contain the following :

- (a) **Proteins of high biological value**, for synthesis of globin (of Hb).
- (b) **Iron**, for synthesis of the heme part of Hb (see iron metabolism).
- (c) Other minerals specially **copper and cobalt**, which act as cofactors in the process of Hb synthesis, and they are required in **trace amounts**.
- (d) **Vitamins** : Almost all vitamins are required particularly **vitamin B₁₂ and folic acid** (*iron and vitamin B₁₂ are the most important nutritional factors required for erythropoiesis*).

IRON METABOLISM

Iron is an **essential element** since it is a component of Hb, myoHb and many enzymes (e.g. cytochrome oxidase). The body contains about **4 gm of iron**, 70 % of which is in Hb, 3 % in myoglobin and the remainder in ferritin (see below).

IRON REQUIREMENTS

The daily requirement of iron **must equal the amount lost**, and is about **0.6 mg in males** and **1.2 mg in females** (due to blood loss in menstruation). However, its usual daily intake is about **20 mg** of which the required amount only is absorbed (i.e. about **3 - 6 % of the ingested amount**).

IRON ABSORPTION

Iron is absorbed mostly in the **duodenum** and only in the **ferrous (Fe²⁺) form** by an **active process**. Iron in heme and in other compounds are both absorbed (figure 13). Its absorption increases when the iron stores in the body are depleted and when erythropoiesis is increased (by an unknown mechanism) and is affected by the following factors :

(1) **Gastric HCl** : The iron in diet is mostly in the **ferric (Fe³⁺)** state. HCl dissolves iron and permits it to form **soluble complexes** with substances that aid its reduction to the Fe²⁺ form (**specially vitamin C**).

(2) **Nature of the iron-containing compound** : Iron in inorganic compounds is more readily absorbed than that in organic compounds.

(3) **Iron salt solubility** : Iron is not absorbed from insoluble salts e.g. iron oxalate and phosphate, and its salts with phytic acid (present in cereals).

(4) **Role of the intestinal mucosal cells (the enterocytes) :**

- Most of iron in the diet is in the ferric (Fe³⁺) form. Fe³⁺ is first converted to Fe²⁺ by the **ferric reductase enzyme** at the brush borders of the enterocytes and Fe²⁺ is then transported into these cells by an apical **membrane iron transporter (DMT 1)**.

- Heme is also transported into the enterocytes by a special **heme transporter (HT)** then the Fe²⁺ in heme is released by the **heme oxygenase enzyme (HO)** and is added to the intracellular Fe²⁺ pool.

- Some of the intracellular Fe^{2+} is converted to Fe^{3+} and combine to a protein called *apoferritin* to form **ferritin** (figure 13).

- The remaining Fe^{2+} is transported to the interstitial fluid at the basolateral borders of the enterocytes by the transporter called *ferroportin 1 (FP)* and such transport is aided by a protein called *hephaestin (Hp)*.

- Fe^{2+} then diffuses into the blood and in the plasma, Fe^{2+} is converted to Fe^{3+} , which becomes bound to the iron transport protein called *transferrin (TF)*. Normally, the plasma iron level is about **130 and 110 microgram %** in males and females respectively.

In many tissues (specially the liver), Fe^{3+} also combines to apoferritin to form ferritin. Ferritin molecules in the lysosomal membranes may aggregate in deposits called **hemosiderin** (that contains as much as 50 % iron). In cases of iron overload, hemosiderin accumulates in the tissues (= **hemosiderosis**) and may lead to **hemochromatosis** (a syndrome that may be hereditary or acquired and is characterized by skin pigmentation, pancreatic damage with diabetes called **bronze diabetes**, liver cirrhosis with a high incidence of hepatic carcinoma, and gonadal atrophy).

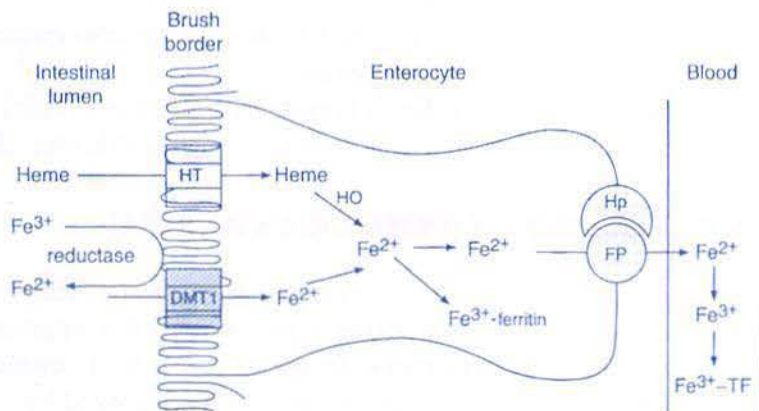


Figure 13 : Mechanism of iron absorption. **HT** = Heme Transporter. **DMT 1** = Apical Membrane Iron Transporter. **HO** = Heme Oxygenase. **FP** = Ferroportin 1. **Hp** = Hephastin **TF** = Transferrin.

VITAMIN B₁₂ (CYANOCOBOLAMIN)

This is a **cobalt-containing vitamin** that is also called **hematinic principle**. It is required together with **folic acid** for red cell maturation (so both are called **erythrocyte maturation factors**) because both are essential for **synthesis of DNA** (therefore, when they are lacking there will be failure of nuclear maturation and division). The daily requirement of vitamin B₁₂ is **1-3 micrograms**, and besides its role in erythrocyte maturation, it also promotes **growth, stimulates other hemopoietic processes** in the bone marrow and is essential for **formation of myelin** in the nervous tissues.

ABSORPTION OF VITAMIN B₁₂

This occurs *in the distal part of the ileum* and it requires a special glycoprotein factor secreted by the *parietal (oxyntic) cells of the stomach*, called the *intrinsic factor*. Vitamin B₁₂ binds to the intrinsic factor and the formed complex is taken up by a lipoprotein present in special receptors in the distal ileum called *cubilin*. This triggers absorption of the complex by *endocytosis*. In the ileal enterocytes, vitamin B₁₂ is released then it is transferred to a special protein called *transcobalamin II* which is absorbed and transports the vitamin in the plasma. *A large amount of vitamin B₁₂ is stored in the liver*, from which it is released to the bone marrow.

CAUSES OF VITAMIN B₁₂ DEFICIENCY

(1) *Lack of the gastric intrinsic factor* (the commonest cause) often due to autoimmune destruction of the parietal cells or after total gastrectomy.

(2) *Certain diseases of the distal ileum* or congenital absence of the specific ileal receptors at which the vitamin is absorbed.

(3) *Intestinal diseases characterized by decreased absorptive power* (= the malabsorption syndromes) e.g. sprue.

****** *Lack of vitamin B₁₂ in diet is very rare*, since it is present in most foods of animal origin and its daily requirement is very little (see above).

VITAMIN B₁₂ DEFICIENCY (PERNICIOUS ANEMIA)

Deficiency of vitamin B₁₂ results in a type of *maturation failure anemia* called *pernicious anemia*. In most cases, deficiency of this vitamin occurs secondary to *absence of the intrinsic factor* as a result of *autoimmune destruction of the parietal cells*. This anemia is characterized by :

(1) Appearance of large red cells called *megaloblasts* in the bloodstream which are *nucleated and larger than mature red cells*. However, they are fragile and their *life span is shorter than normal*, so they hemolyze easily and the resulting anemia is called *megaloblastic or macrocytic anemia*. The Hb content in the megaloblasts is greater than normal but the mean corpuscular Hb concentration is normal (see blood indices below).

(2) *Leukopenia and thrombocytopenia* due to less synthesis of DNA.

(3) *Neurological symptoms* : These occur due to degeneration of the dorsal and lateral columns of the spinal cord as well as the peripheral nerves, a condition known as *subacute combined degeneration*.

Treatment of pernicious anemia

Administration of vitamin B₁₂ by injection and not by mouth (because most cases occur secondary to lack of the intrinsic factor).

FOLIC ACID (PTEROYLGLUTAMIC ACID)

This is also an *erythrocyte maturation factor* the daily requirement of which is about **400 micrograms (0.4 mg)**. It is absorbed mostly in the jejunum and it is changed in the body to a more active substance called *folinic acid*. Its lack also results in *maturation failure macrocytic anemia* and may be due to (a) Deficient supply in diet (b) Increased requirements e.g. during pregnancy (c) Failure of absorption (e.g. in steatorrhea).

Folic acid *can improve cases of pernicious anemia, but it may increase the neurological lesions* because it shifts vitamin B₁₂ from the nervous tissues to the bone marrow.

THE ANEMIAS

Anemia is a *pathological condition* characterized by lowering of the circulating red cell mass below the normal level or reduction of the Hb concentration (or both together), leading to *a decrease in the O₂ carrying capacity of the blood*. Generally, it is due to either *a decrease in the production or an increase in the destruction of the red blood cells*, and it may be classified in one of the following 2 ways :

(A) According to the cause

(1) **Hemorrhagic anemia** : This is anemia caused by acute or chronic *blood loss* (e.g. due to bleeding peptic ulcers or piles, or *bilharziasis*).

(2) **Hemolytic anemia** : This is anemia caused by excessive hemolysis (breakdown) of the R.B.C.s. Hemolysis may occur due to either :

(a) **Intracapsular (congenital) causes** e.g. abnormalities in the red cell membranes (as in congenital spherocytosis) or in Hb (as in sickle cell anemia and thalassemia) or deficiency of the G6PD enzyme.

(b) **Extracapsular (acquired) causes** e.g. certain drugs, chemicals and toxins (as some snake venoms), certain infections, malaria, hypersplenism, incompatible blood transfusion and autoimmunity (i.e. formation of abnormal antibodies that attack the red blood cells).

(3) **Aplastic anemia** : This is anemia caused by *damage of the bone marrow* e.g. due to (a) Exposure to excessive x ray or gamma ray radiation (b) Certain drugs (e.g. chloramphenicol) and chemicals (e.g. arsenic) (c) Leukemia (in which the excessively-proliferating white cells in the bone marrow decrease red cell formation) (d) Certain chronic infections.

(4) **Nutritional (deficiency) anemias** : This is anemia caused by deficiency of the nutritional factors that are required for erythropoiesis e.g. :

(a) Anemia due to deficient supply of iron (iron deficiency anemia).

(b) Pernicious anemia (due to vitamin B₁₂ deficiency).

(c) Macrocytic anemia (due to folic acid deficiency).

(B) According to red cell size and Hb concentration

(1) **Normochromic normocytic anemia** : In this anemia, the size of the red blood cells and their Hb content are *normal* but their count is less than normal. It occurs in (a) Hemorrhagic and hemolytic anemias (c) Some cases of aplastic anemia (d) Hepatorenal disease (e) Chronic infections.

(2) **Hypochromic microcytic anemia** : In this anemia, the red cell size and their Hb content are decreased but their count is almost normal. It occurs in (a) Iron deficiency anemia (b) Thalassemia (c) Defective synthesis of porphyrin and heme e.g. in lead intoxication (d) Hypothyroidism.

(3) **Macrocytic anemia** : In this anemia, both the red cell volume and its Hb content are increased but the mean Hb concentration is almost normal (see blood indices). There are 2 types of this anemia :

(a) **Megaloblastic (maturation failure) anemia** : This occurs in *pernicious anemia and in cases of folic acid deficiency*.

(b) **Non-megaloblastic anemia** : This occurs after recent hemorrhage or hemolysis and in some cases of aplastic anemia as well as in some cases of anemia resulting from hepatic disease.

****** : The *commonest cause of iron deficiency anemia in Egypt* is chronic blood loss as a result of *bilharziasis*. This type of anemia also occurs *in infants* if iron is not supplied after the 6th month of age (page 32) and *in females* if the iron lost during menstruation is not compensated.

EFFECTS OF ANEMIA

(1) **Reduction of the blood viscosity and O₂ lack**. The former decreases the peripheral resistance while the latter causes V.D. Both effects increase the venous return and consequently the cardiac output, which *increases the work load on the heart*.

(2) **Increase of the blood velocity** (due to reduction of viscosity) and this *increases the blood turbulence* which accentuates the heart sounds and may produce *functional murmurs* (refer to circulation).

(3) **Decrease of the O₂ content in the arterial blood** which leads to *tissue hypoxia and rapid fatigue*.

THE BLOOD INDICES

(1) **Colour Index (CI)** : This is a rough (approximate) indication of *the amount of Hb in the red cells*. It is calculated as follows :

$$CI = \frac{\text{Hb content (\% of normal)}}{\text{Red cell count (\% of normal)}} \quad \text{Its normal value is } \frac{100\%}{100\%} = 1$$

(*normal range 0.85 - 1.1*). Lower values suggest decreased Hb content, most commonly as a result of iron deficiency.

(2) **Mean Corpuscular Hb (MCH)** : This is the average amount of Hb in one red blood cell in picograms (pg) . It is calculated as follows :

$$\text{MCH} = \frac{\text{Hb content in one litre blood}}{\text{Red cell count in millions/mm}^3} . \text{ Its normal value is } \frac{150}{5} = 30 \text{ pg}$$

(*normal range is 26 to 34 pg*). Higher values (up to 38 pg) are found in macrocytic anemias while lower values are obtained in microcytosis and when Hb synthesis is decreased or defective e.g. in hypochromic anemias.

(3) **Mean Corpuscular Hb Concentration (MCHC)** : This is the average amount of Hb per 100 ml of red cells. It is calculated as follows :

$$\text{MCHC} = \frac{\text{Hb (gm) in 100 ml blood}}{\text{Packed cell volume}} \times 100 . \text{ Its normal value is } \frac{15}{45} \times 100 = 33\text{-}35 \text{ gm /100 ml red cells (= normochromic cells). Below 31 gm, the cells are hypochromic. In macrocytic anemia there is normochromia .}$$

(4) **Mean corpuscular volume (MCV)** : This is the average volume of one red blood cell. It is calculated as follows :

$$\text{MCV} = \frac{\text{Packed cell volume / one litre blood}}{\text{Red cell count in millions/mm}^3} . \text{ Its normal value is } \frac{450}{5} = 90 \text{ microns}^3 \text{ ranging from 78 to 94 microns}^3 (= \textit{normocytes}). \text{ Red blood cells with more volumes are } \textit{macrocytes} \text{ (e.g. in pernicious anemia) while those with less volumes are } \textit{microcytes} \text{ (e.g. in iron deficiency anemia).}$$

CHAPTER 6

BLOOD TYPES (GROUPS) AND BLOOD TRANSFUSION

The outer layers of the red cell membranes contain certain *antigens* which are chemically *glycosphingolipids* and called *agglutinogens* because they cause red cell agglutination under certain conditions (see below).. There are 2 types of such antigens called *A and B agglutinogens*, and according to the present type, the human blood is divided into 4 groups :

1. **Group A** (about 42 %) when only type A is present.
2. **Group B** (about 9 %) when only type B is present.
3. **Group AB** (about 3 %) when both types A and B are present.
4. **Group O** (about 46 %) when both types A and B are absent.

The plasma contains certain gamma globulins called *agglutinins* which are *antibodies to the red cell agglutinogens* and are also 2 types : *anti-A (or alpha) agglutinins and anti-B (or beta) agglutinins*.

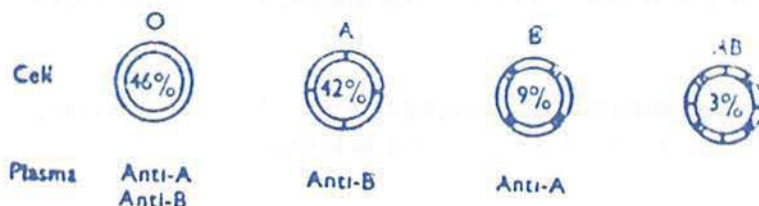


Figure 14 : The various human blood groups and their frequency %

Agglutinins begin to be produced within a few months after birth and *infants develop agglutinins against the agglutinogens not present in their own red cells*. Accordingly, type A subjects develop anti-B agglutinins, type B subjects develop anti-A agglutinins, type O subjects develop both, and type AB subjects develop neither (figure 14).

Therefore, *agglutinogens normally never exist with their corresponding agglutinins*, otherwise an *antigen antibody reaction* that leads to the dangerous process of *red cell agglutination* would occur. The latter occurs in cases of *incompatible (= mismatched) transfusions* in which the agglutinins are attached to the red cells, leading to their *adherence and clumping* (figure 16). These clumps plug small blood vessels then they are destroyed by the phagocytic leukocytes and reticuloendothelial system resulting in *hemolysis*.

****** There are 2 types of agglutinin A called *A₁ and A₂*. However, the difference between both types is only *quantitative* (each *A₁* red cell has about one million copies of the A antigen while each *A₂* red cell has only about 1/4 million). Accordingly, the ABO system of blood groups contains *6 types and not 4*, namely groups *A₁, A₂, B, O, A₁B and A₂B*.

IMPORTANCE OF BLOOD TYPING IN BLOOD TRANSFUSION

In blood transfusion, *the red cells that may agglutinate are those of the donor*. Therefore, it is *essential that the recipient's plasma should not contain agglutinins against the donor's red cells*. The agglutinins in the plasma of the donor usually have no ill effects on the recipient's red cells because they are diluted by the much larger volume of the recipient's plasma. However, *this is not a rule*, and therefore, *intra-group transfusions are preferred*, and if this is not possible, a *double cross matching test* should be performed before transfusion (see below).

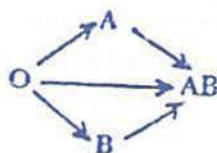


Figure 15 : Possible transfusions among various blood groups.

According to these principles, blood group AB (containing no agglutinins in its plasma) is a **universal recipient** while blood group O (containing no agglutinogens in its red cells) is a **universal donor** and the possible transfusions among various groups are as follows (figure 15).

- Group AB is a universal recipient and gives only group AB.
- Group O is a universal donor and receives blood only from group O.
- Group A can receive blood from groups A & O and gives blood to groups A & AB.
- Group B can receive blood from groups B & O and gives blood to groups B & AB.

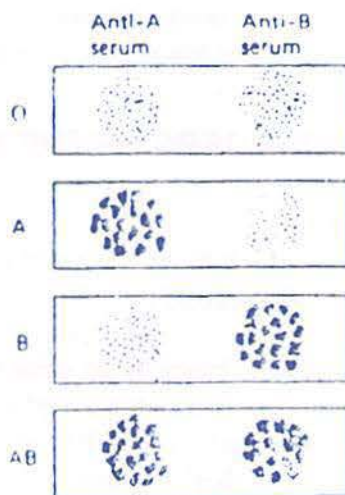


Figure 16 : Blood grouping (typing) by the slide technique.

BLOOD TYPING (DETERMINATION OF THE BLOOD GROUPS)

This is usually carried out by the *slide technique* (figure 16). The blood under test is diluted with isotonic saline, and 2 drops are placed on a glass slide. Anti-A serum is then mixed with one of the drops while anti-B serum is mixed with the other. After 2-3 minutes, the drops are examined for an antigen-antibody reaction, as denoted by red cell agglutination (i.e. clumping), and the results are interpreted as follows :

- *If agglutination occurs with both sera, the subject is group AB*
- *If agglutination occurs with anti-A serum only, the subject is group A.*
- *If agglutination occurs with anti-B serum only, the subject is group B.*
- *If no agglutination occurs with both sera, the subject is group O.*

****** In addition to the classical ABO system of agglutinogens, the human red cells also normally contain many other antigenic systems, and it is said that *there are over 500 billion blood group phenotypes*. However, the *Rh and M & N antigens* are of special clinical importance.

THE Rh ANTIGEN SYSTEM

This is the system of agglutinogens normally present in the red cells of *Rhesus monkeys* (hence the name *Rh*), of which there are 6 varieties known as the *C, D and E antigens*. These antigens were discovered in the human red cells, and *antigen D has the strongest antigenic effect*. The term *Rh+ve* means that the individual has *agglutininogen D* (and vice versa) and the Rh typing serum used in routine blood typing is *anti D serum*.

About 85 % of the population are D (or Rh) +ve while the remaining 15 % are D -ve. However, *D -ve individuals do not normally have D agglutinins (= antibodies) in their plasma* and these antibodies are formed *only if they receive D +ve blood* (because the Rh factor behaves as a foreign antigen in Rh-ve persons).

IMPORTANCE AND DANGERS OF THE Rh ANTIGEN

(1) Transfusion reactions

If Rh-ve individual receives Rh+ve blood, he will develop Rh agglutinins in his plasma, and a second transfusion with Rh+ve blood leads to agglutination then hemolysis of the transfused red cells

(2) Erythroblastosis fetalis (hemolytic disease of the newborn)

This is a serious disease that can *affect Rh+ve fetuses* if the mother was Rh-ve and sensitized by the Rh antigen. In this case, the formed Rh agglutinins in the mother's plasma can easily diffuse across the placenta to the fetus (*due to their small size*) resulting in agglutination and hemolysis of his red cells. The fetus may die in utero or born with severe

hemolytic anemia and jaundice (due to excessive bilirubin formation). As a compensation, immature erythroblasts are rapidly delivered from the bone marrow to the bloodstream (hence the name, *erythroblastosis fetalis*). In addition, the liver may be severely damaged, the fetus may be grossly edematous (= *hydrops fetalis*), and brain damage (specially in the basal ganglia) may occur as a result of excessive bilirubin deposition leading to a serious condition called *kernicterus*.

How may the Rh-ve mother be sensitized by the Rh antigen ?

(1) *By previous transfusion with Rh+ve blood at any time (even during childhood).* In this case, *her first baby may suffer the disease.*

(2) *When she marries Rh+ve man and becomes pregnant in a Rh+ve fetus.* In some of these cases, the fetal Rh+ve red cells may leak into the maternal circulation leading to formation of Rh agglutinins in her plasma, which subsequently cross the placenta to the fetus resulting in agglutination then hemolysis of his red cells. Entrance of the Rh+ve fetal red cells into the maternal circulation during pregnancy may occur as a result of fetal movements or fetal-maternal hemorrhage. However, it occurs *mostly at the time of delivery*, so the *fetus of the first pregnancy is usually born normal*, but serious effects often occur in *the fetuses of the second or third pregnancies.*

Avoidance and treatment of erythroblastosis fetalis

To avoid erythroblastosis, *Rh-ve females should not be transfused with Rh+ve blood and should not marry Rh+ve men* (if possible). Also, if a Rh-ve mother delivers a Rh+ve baby, she must be given a single dose of *anti-Rh antibodies (Rh immune globulin)* early in the postpartum period after labor (which prevents formation of Rh agglutinins in her plasma).

On the other hand, an erythroblastic baby can be saved (if born alive) by *repeated exchange blood transfusion with Rh-ve blood* during the first few weeks of his life.

BLOOD TRANSFUSION

TYPES OF BLOOD TRANSFUSION

(1) HETEROLOGOUS TRANSFUSION

This is transfusion of *foreign blood* (i.e. from another person).

(2) AUTOLOGOUS TRANSFUSION

This is transfusion of the *patient's own blood*. Blood is withdrawn from the patient before a surgical operation, then it is transfused back to him (if a transfusion is needed). This becomes recently popular due to *fear of transmission of AIDS* and also because it *eliminates the risks of the heterologous transfusion reactions* (see below).

INDICATIONS FOR BLOOD TRANSFUSION

- (1) After hemorrhage, to restore the blood volume.
- (2) Severe anemia, to increase the O₂ carrying capacity of the blood.
- (3) Hemophilia and parahemophilia, to supply the missing clotting factors
- (4) Purpura, to increase the platelet count..
- (5) Leukopenia, specially agranulocytosis, to increase the white cell count
- (6) Severe infections, to increase immunity by supplying gamma globulin.
- (7) Erythroblastosis fetalis (see above).
- (8) Hypoproteinemia, to increase the amount of plasma proteins.
- (9) Chronic leukemia (if there is severe anemia or thrombocytopenia).

PRECAUTIONS BEFORE BLOOD TRANSFUSION

- (1) The transfused blood should be *compatible with that of the recipient*. ABO and Rh compatible blood is clinically-acceptable for transfusion.
- (2) The Hb content of the transfused blood *must not be less than 90 %*.
- (3) The transfused blood should be *free from diseases* (e.g. infective hepatitis, malaria and AIDS).
- (4) The transfused blood must *be fresh and not frozen* (it must be stored at 4 °C and for a period not exceeding 21 days).
- (5) A *cross matching test* should be done (see next).

CROSS MATCHING TEST

This is a simple *pre-transfusion compatibility test* that should be done since it is the best safeguard against the complications of blood transfusion (see below). It is performed by matching the *donor's red cells against the recipient's plasma directly*. It is also advisable to match the recipient's red cells against the donor's plasma (= *double cross machine*). Agglutination in any of these tests indicates incompatibility.

EFFECTS OF INCOMPATIBLE BLOOD TRANSFUSION

Incompatible (mismatched) blood transfusion leads to *agglutination of the donor's red cells* followed by their hemolysis. This occurs due to an *antigen-antibody reaction* between the red cell antigens and their plasma antibodies, and results in the following effects :

- (1) *Severe pain* elsewhere in the body due to blocking of the capillaries by the clumps of agglutinated red cells.
- (2) *Hemolytic jaundice* due to excessive bilirubin formation from the Hb released from the hemolyzed red cells.
- (3) *Dyspnea, chest pain, chills and fever*.
- (4) *Hypotension (and may be shock)* as a result of the generalized V.D.

caused by the released *histamine* from the hemolyzed red cells.

(5) **Hyperkalemia** due to release of K^+ from the hemolyzed red cells and retention of K^+ in the body as a result of renal failure (see next).

(6) **Oliguria or anuria** due to hypotension(see above) and hemoglobinuria (Hb is precipitated in the renal tubules leading to their blockage). Such effects may result in acute renal failure..

COMPLICATIONS (DANGERS) OF BLOOD TRANSFUSION

- (1) **Incompatibility** due to mismatched blood groups (see above).
- (2) **Allergic reactions** : The antigen-antibody reactions do not only cause red cell agglutination but also other effects, specially fever due to release of certain pyrogenic substances.
- (3) Bacterial infections (in case of unhygienic transfusion procedures).
- (4) Transmission of diseases (e.g. AIDS and hepatitis).
- (5) Massive blood transfusion may lead to **circulatory overload, hyperkalemia or citrate toxicity**.

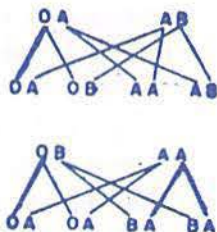


Figure 17 : Inheritance of the blood groups.

INHERITANCE OF BLOOD GROUPS

The A, B and O antigens are inherited, and the person's blood group is determined by the 2 antigens which he receives from his parents. The *A and B antigens are inherited as dominant Mendelian allelomorphs* while the *O antigen is recessive*. Therefore, a person having a **phenotype A** (i.e. group A) may be one of the following **genotypes** :

1. **Genotype AA (homozygous)** if he inherited an A antigen from each parent

2. **Genotype AO (heterozygous)** if he inherited an A antigen from one parent and an O antigen from the other.

Similarly, phenotype B may be homozygous (genotype BB) or heterozygous (genotype BO) while phenotype O is only homozygous (genotype OO).

The D (Rh) antigen is also inherited. *There are 2 types of this antigen* : **D antigen** which is dominant and **d antigen** which is recessive. D+ve persons may be homozygous (genotype DD) or heterozygous (genotype Dd) while D-ve persons are only homozygous (genotype dd).

Due to blood group inheritance, the *genotype of a fetus can be predicted if the genotypes of his parents are known* e.g. if the father had a genotype AO and the mother AB, the genotypes of their children would be either AO, BO, AA or AB. Also, if the genotypes of the parents were BO and AA, the genotypes of their children would be either AO or AB (figure 17). Similarly, if the father had genotype Dd and the mother dd, their children would be either Dd (D+ve) or dd (D-ve).

IMPORTANCE OF DETERMINATION OF BLOOD GROUPS

- (1) *In blood transfusion (to avoid blood group incompatibility).*
- (2) *In marriage (to avoid erythroblastosis fetalis).*
- (3) *Medicolegal importance :*

Determination of the blood groups is commonly used in investigating cases of *disputed paternity*. However, blood grouping tests *can only exclude paternity but cannot prove it* e.g. if a child had a genotype BB, and his mother had a genotype BO, a man having a genotype AA, AO or OO cannot be his father, but if the man had a genotype AB, BB or BO, he may be the father of the child (but it is not certain that this man is the father because thousands of other people have these genotypes).

However, paternity can still be proved (or excluded) by (1) Determination of other inherited red cell antigens (specially the *D and M and N antigens*) (2) Determination of the *human leukocyte antigen* (= HLA) (page 62). (3) *DNA examination* in the child and the claimed father.

CHAPTER 7

DEFENCE MECHANISMS OF THE BODY

The body is protected against pathogenic organisms by 2 mechanisms

- (1) **Phagocytosis of invading organisms** : This is carried out by the white blood corpuscles or cells (W.B.C.s) and the reticuloendothelial system.
- (2) **Formation of antibodies** against the invading organisms or destroying them by lymphocytes through the *immune response* (= *immunity*).

THE WHITE BLOOD CELLS (LEUKOCYTES)

These are **large nucleated cells** that constitute the *mobile units* of the body's defence system (because they seek and destroy foreign invaders).

Their total count is **4000 - 11000 / mm³** and they include the following :

(A) Granulocytes (polymorphonuclear leukocytes) : These include

- (1) Neutrophils or microphages (50-70 %)
- (2) Eosinophils (1-4 %).
- (3) Basophils (0.4 %).

(B) Non-granulocytes : These include :

- (1) Lymphocytes (20 - 40 %).
- (2) Monocytes or macrophages (2 - 8 %).

LEUKOPOIESIS (FORMATION OF LEUKOCYTES)

All granulocytes and monocytes (as well as a few lymphocytes) are formed in the **bone marrow**, while most lymphocytes are formed in the **lymphoid organs** (specially the lymph nodes and spleen). Leukopoiesis requires **all the dietary factors necessary for cell growth and division** (e.g. vitamin B₁₂ and proteins of high biological value), and is stimulated by certain **colony-stimulating factors (CSFs)** that are produced by the macrophages, fibroblasts, endothelial cells and certain lymphocytes. They **act locally in the bone marrow**, and each CSF stimulates proliferation of **particular committed stem cells**.

LIFE SPAN AND FATE OF LEUKOCYTES

(1) The life span of **granulocytes** in the circulation is 4-8 hours, after which they enter the tissues and stay 4-5 days then they are destroyed, and some are lost from the body via the GIT. In infections, their total life span is much shortened because they are attacked and destroyed by the invading organisms (forming **pus cells**).

(2) The life span of **monocytes** in the blood is 20 - 72 hours, after which they enter the tissues and enlarge into **tissue macrophages** which live for long periods (3 months or more).

(3) **Large lymphocytes mature into small lymphocytes** which reach the bloodstream via the **lymphatic vessels**. Then, after a few hours they cross the capillary walls to the tissues, from which they **re-enter the lymphatic vessels and circulate again** thus they live for relatively long periods.

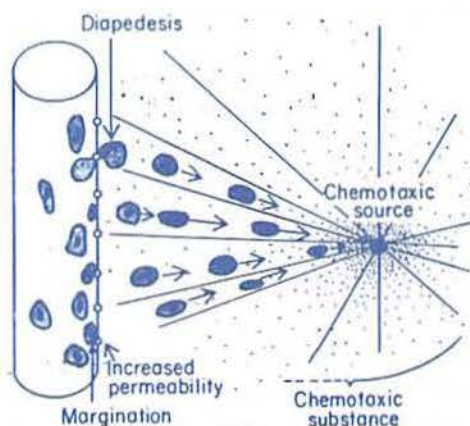


Figure 18 : Movement of neutrophils toward areas of infection.

FUNCTIONS OF LEUKOCYTES

(A) NEUTROPHILS (MICROPHAGES)

These exert a powerful *defence action* against bacterial infections. Their *main function is phagocytosis and destruction of the invading bacteria*. When an infection occurs, the macrophages present in the tissues attack the bacteria and within the first hour, neutrophils invade the inflamed area (thus, the tissue macrophages are the *first line of defence against bacterial infections while neutrophils are the second line*). This is associated by a marked increase of the number of neutrophils in the blood (= *neutrophilia*) as a result of stimulation of the bone marrow to release large numbers of neutrophils (by certain products of inflammation that enter the bloodstream). The neutrophils reach the site of infection by the following steps (figure 18) :

- (1) They are first attracted to the capillary endothelial surface by proteins called *selectins* and they roll along it. This effect is called *margination*.
- (2) They bind firmly to a protein (*integrin*) adhesion molecules, then they insinuate themselves through the capillary walls between the endothelial cells by a process of *diapedesis* and enter the tissue spaces.
- (3) They actively move toward the site of infection by an *ameboid motion*.
- (4) They are attracted to the infected area by many chemical substances. This phenomenon is called *chemotaxis* and many *chemotactic agents* (= *chemokines*) are known, the most important of which are (a) A component of the complement system (see below) (b) Polypeptides from lymphocytes, mast cells and basophils (c) Some bacterial or viral toxins, and the degenerative products of inflamed tissues (d) *Leukotrienes* (see below).

Mechanism of action of neutrophils

(1) Certain plasma factors coat the bacteria making them "tasty" to the phagocytes. This process is called *opsonization* and the main *opsonins* are certain complement proteins as well as immunoglobulin G (see later).

(2) The coated bacteria bind to receptors on the neutrophil cell membranes, then they are ingested inside these cells by *endocytosis* (forming phagocytic vacuoles in their cytoplasm called *phagosomes*).

(3) The cytoplasmic granules come in contact with the phagosomes and discharge their contents into them (= *degranulation*). These granules contain (a) Protease (proteolytic) enzymes that digest bacteria (b) Anti-microbial proteins called *defensins* (c) The *myeloperoxidase enzyme*. This enzyme catalyzes the reaction between H_2O_2 and Cl^- to form HOCl (hypochlorite), which is a powerful bactericidal substance.

(4) The enzyme *NADPH oxidase* (which is formed by the organelles called *peroxisomes* and is bound to the phagosomal membranes) is also activated. This enzyme catalyzes formation of *superoxide* ($= \text{O}_2^-$, a free radical formed by addition of one electron to O_2). 2O_2^- react with 2H^+ to form H_2O_2 by activity of the *superoxide dismutase enzyme*. Both O_2^- and H_2O_2 are oxidants that are effective bactericidal agents.

****** The neutrophils also release the *platelet activation factor* (= PAF, page 17) and *leukotrienes* (substances that increase capillary permeability) which are also secreted by the basophils and mast cells (see in immunity).

(B) EOSINOPHILS

Like neutrophils, eosinophils have a short life in the circulation and are *attracted to the endothelial cells by selectins and bind to integrins* then they enter tissues by *diapedesis*. They are specially abundant in the GIT mucosa (where they defend against parasites) as well as in the mucosa of the respiratory and urinary tracts. They are also *phagocytic and exhibit chemotaxis*, but they are less motile and weaker than neutrophils. They are produced in large numbers in the following conditions :

(1) *Parasitic infections* (e.g. bilharzia), in which the eosinophils attack the parasites (specially the juvenile forms) and kill them by hydrolytic enzymes and highly reactive forms of O_2 that are released from their granules.

(2) *Allergic diseases* (e.g. bronchial asthma) in which the eosinophils are attracted to the sites of allergic reactions (by a chemotactic factor released from the basophils and mast cells) where they decrease and prevent spread of the allergic inflammatory process by (a) Detoxication of some inflammation-inducing substances released by the basophils and mast cells (b) Phagocytosis and destruction of the allergen-antibody complexes.

(C) BASOPHILS

These are *non-phagocytic* cells that resemble the *mast cells*. Both form and liberate *heparin and histamine and other inflammatory mediators* (e.g. *leukotrienes*) as well as small amounts of *bradykinin and serotonin*. These substances produce the local vascular and tissue reactions that occur in *allergic conditions*, and are responsible for the immediate-type hypersensitivity reactions which may be as mild as urticaria and rhinitis or as severe as an anaphylactic shock

(D) MONOCYTES (MACROPHAGES)

Monocytes in the blood enter the tissues and become the *tissue macrophages*. These are the main constituent of the *reticuloendothelial system* (see below) and are the *first line of defence against bacterial infection* (the neutrophils are the second line). In cases of severe inflammation, they are released into the bloodstream from the bone marrow in large numbers then they move to sites of infection by *diapedesis, amoeboid motion and chemotaxis* where they phagocytize and kill bacteria by similar mechanisms as those of neutrophils. Such process requires a few days to develop, so it is *slower than the movement of neutrophils* and represent a *third line of defence against bacterial infection*. However, the effects of monocytes are *much more powerful than neutrophils*.

In addition, monocytes engulf necrotic tissue and play an important role in *immunity* (see below).

(E) LYMPHOCYTES

These are the major constituent of the *immune system* (see below).

VARIATIONS OF THE LEUKOCYTIC COUNT

(A) PHYSIOLOGICAL VARIATIONS

(1) **Diurnal variation** : The leukocytic count shows continuous irregular fluctuations during the 24 hours of the day, being lowest in the morning and highest in the evening.

(2) **Age** : The leukocytic count is higher than normal in newly born infants in whom the *lymphocytes constitute about 50 % of the total count*.

(3) **Physiological leukocytosis** : This occurs in pregnancy, after a cold bath and muscular exercise, following meals and in most emotions.

(B) PATHOLOGICAL VARIATIONS

(1) **Pathological leukocytosis :** (a) *Neutrophilia* occurs in cases of tissue damage (e.g. myocardial infarction) and pyogenic infections (e.g. pyelitis and tonsillitis) (b) *Eosinophilia* occurs in allergic and parasitic diseases (c) *Basophilia* occurs in allergic conditions (d) *Lymphocytosis* occurs in tuberculosis and whooping cough (e) *Monocytosis* occurs in malaria.

(2) **Leukemia :** This is a malignant disease of the bone marrow or lymphoid tissue in which there is *uncontrolled leukopoiesis* (producing *mye-logenous or lymphocytic leukemia* respectively), so that the leukocytic count may increase to $500000/\text{mm}^3$ or more. The condition causes (a) Bone pain and easy fractures (in the myelogenous type). (b) Spread of the leukemic cells in the body causing extensive tissue destruction everywhere (c) Severe anemia and bleeding tendency (due to replacement of the red cell and platelet forming cells in the bone marrow by the nonfunctional abnormal leukemic cells) (d) Excessive use of the body metabolic substrates by the growing cancerous cells, which depletes the energy of the patient and leads to death.

(3) **Leukopenia and agranulocytosis :** Leukopenia might occur in *chronic infections* as a result of bone marrow depression. On the other hand, agranulocytosis is a condition of *bone marrow aplasia* in which the bone marrow almost stops producing leukocytes and other blood cells (see aplastic anemia). It is a dangerous condition because it leaves the body unprotected against bacteria and other invading organisms, and it commonly follows radiations by gamma rays (e.g.- due to a nuclear explosion) or extensive use of certain drugs (e.g. chloramphenicol).

THE RETICULOENDOTHELIAL SYSTEM (Tissue Macrophage System)

This system consists mainly of the *tissue macrophages* (which are originally *monocytes* that have entered from the blood) and few specialized endothelial cells in *the bone marrow, spleen and lymph nodes*. Its main functions are the following :

(1) **Defence :** The tissue macrophages attack and phagocytize the invading organisms in the following sites :

(a) *Skin and subcutaneous tissue* (by macrophages called *histiocytes*).

(b) *Lungs* (in which the bacteria and foreign particles e.g. dust, carbon and silica are engulfed by the *pulmonary alveolar macrophages*).

(c) *Liver* (in which the *Kupffer cells* attack the bacteria that continuously pass from the GIT).

(d) *Lymph nodes* (in which the macrophages attack the bacteria transmitted from the tissues).

- (e) *Bone marrow and spleen* (in which the macrophages attack the bacteria that enter the blood stream).
- (2) **Removal of all old blood cells.**
- (3) **Breakdown of Hb, formation of bile pigments and storage of iron.**
- (4) **Repair of damaged tissues after inflammation** (by engulfing the necrotic tissue and releasing a *tissue-growth factor* by the macrophages).
- (5) **Extramedullary hemopoiesis** (in cases of severe bone marrow depression, the endothelial cells in the spleen can form blood cells).
- (6) **Some erythropoietin is formed by the liver Kupffer cells** (page 41)

FUNCTIONS OF THE SPLEEN

- (1) **Defence** : This is produced by (a) The large number of macrophages in the spleen (b) Formation of lymphocytes and plasma cells which play a major role in immunity (see below). Therefore, in absence of the spleen, the bacterial infections become more common and more severe.
- (2) **Blood cell formation** (red cells in the fetus and lymphocytes in adults). It is also the main site of extramedullary hemopoiesis in adults (see above)
- (3) **Blood cell removal** : All old and abnormal blood cells (e.g. spherocytes) are removed by the macrophages in the spleen, and the Hb released from the red cells is degraded leading to formation of *bile pigments* while the iron is stored (see above). Excessive platelet breakdown in the spleen (= *hypersplenism*) may be a cause of thrombocytopenic purpura (page 29).
- (4) **Blood storage** : The human spleen can accommodate about 50 ml of concentrated blood in its sinuses. This blood can be added to the bloodstream by contraction of the smooth muscle in the splenic capsule through excitation of its sympathetic nerve supply (which specially occurs in cases of O₂ lack e.g. after hemorrhage). However, this function is *not important* because the stored amount of blood is small and the contraction of the splenic capsule is relatively weak.

IMMUNITY

Immunity is the ability of the body to resist various microorganisms and toxins that may damage the tissues or organs, and it is **2 types** :

- (1) INNATE (NATURAL) IMMUNITY** : This is produced mainly by :
 - a- The *natural resistance of the skin* to invasion by various organisms.
 - b- Phagocytosis of bacteria and other invaders by the *white blood cells, the tissue macrophage system and the natural killer cells* (page 66).
 - c- Destruction of many swallowed organisms by the *gastric HCl*.
 - d- Presence of certain chemical compounds in the blood that attack and destroy foreign organisms e.g. the *lysozymes and complement complex*.

(2) ACQUIRED (ADAPTIVE) IMMUNITY : This is the development of an *immune response* against foreign organisms or toxins once they enter the body by the activity of a special system called the *immune system*. The basic constituents of this system are the *lymphocytes*, and the immune response occurs by activation of these cells to attack the invaders (which are called *antigens*) either *directly or by forming antibodies* against them.

Most antigenic substances are either *proteins or polysaccharides* but antibodies can also be formed against nucleic acids and lipids if these are presented as nucleoproteins and lipoproteins.

Normally, the immune system do not attack the body's own tissues (= *self or immune tolerance*). However, sometimes such tolerance fails and an immune response develops against the body's own tissues resulting in a group of diseases known as the *autoimmune diseases* (page 67).

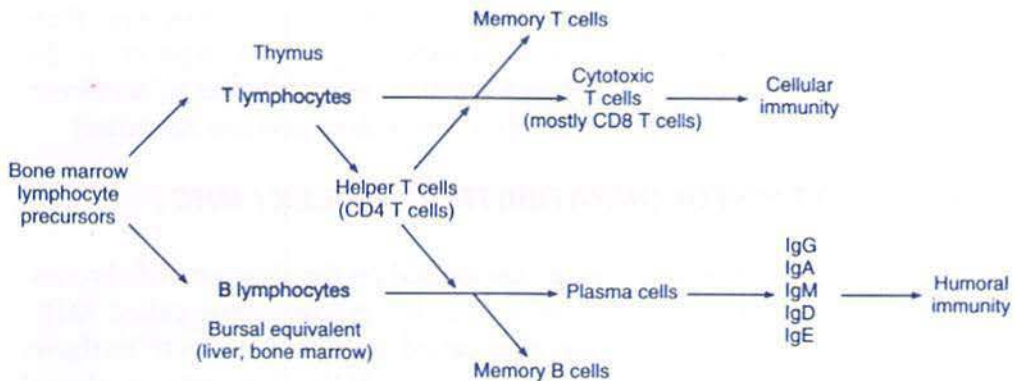


Figure 19 : Development of the immune system and types of lymphocytes

THE IMMUNE SYSTEM

The immune system consists mainly of the *lymphocytes*. The lymphocyte precursors *are delivered from the bone marrow to certain organs in which they are processed (become mature), then they migrate to the lymphoid organs..* These organs are the following (figure 19) :

(1) **The thymus :** Lymphocyte precursors are processed in this organ into a type of lymphocytes called the *T-lymphocytes*, which further differentiate into the following 3 types :

(a) **Helper-T cells** (or CD4 T cells, because they have on their surfaces a glycoprotein called CD4). There are 2 types : *helper-1 & helper-2 T cells*.

(b) **Cytotoxic T cells** (or CD8 T cells because they have the glycoprotein CD8). *There are also 2 types of the cytotoxic T cells.*

(c) **Memory T cells** (which are silent cytotoxic T cells).

(2) **The liver in the fetus and bone marrow in adults :** Lymphocyte precursors are processed in these organs into another type of lymphocytes called the *B- lymphocytes*. In birds, the B-lymphocytes are processed in a lymphoid structure near the cloaca called the *bursa of Fabricius* (B refers to this bursa), but since this bursa is absent in humans, procession of the B- lymphocytes occurs in the liver (and after birth in the bone marrow), so these organs are called *bursal equivalents*. The B lymphocytes further differentiate into *plasma cells and memory B cells*

****** Both the T and B lymphocytes are *morphologically similar* but they can be identified by certain markers on their cell membranes.

****** The number of antigens that can be recognized by lymphocytes is extremely large, and there are many million different types of B and T lymphocytes, each with the ability to respond to a particular antigen.

****** The *memory T and B cells are inactive* and persist in the body for months, years or even life-long (*as in case of measles*). However, these cells can be converted to effector lymphocytes by a later exposure to the same antigen that have caused their formation, in which case an *accelerated and greater response is produced* (= *Secondary immune response*).

THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

The MHC refers to special genes located on the short arm of chromosome 6 . They encode formation of certain glycoproteins called MHC proteins (or antigens), and are also called **human leukocyte antigens (HLA)**. There are 2 types of these proteins, *MHC-I protein* (= *class I MHC proteins or antigens*) and *MHC-II protein* (= *class II MHC proteins or antigens*). *The antigen presenting cells contain both types* (see below), *while all other cells in the body contain MHC-I protein only*.

TYPES OF ACQUIRED IMMUNITY

There are 2 types of immunity known as *humoral and cellular immunity* which are produced by the **B and T lymphocytes respectively**.

(A) HUMORAL IMMUNITY (B-CELL IMMUNITY)

This is produced by the *B- lymphocytes* (figure 19), and is a major defence against *bacterial infections*. When these cells are activated by antigens they proliferate and differentiate into *plasma cells and memory-B cells*. The plasma cells secrete antibodies called *immunoglobulins* into the lymph, which then pass to the bloodstream, circulate in the *globulin fraction of the plasma proteins* and attack the invading antigens.

Mechanism of humoral immunity

The B-cells *can be stimulated to secrete immunoglobulins by direct contact with the antigens*. However, *contact with helper-2 T cells is essential for their full activity*. This occurs as follows :

(1) Antigens are engulfed by the *antigen presenting cells (APCs)* which are mainly the *macrophages and the dendritic cells* (which are present all over the body specially in the lymph nodes, spleen and skin).

(2) In the APCs, the peptide products of antigen digestion are coupled to the *MHC-II* proteins and are presented on the cell surface

(3) The APCs come in contact with *CD4 helper 2-T cells* and at specific *antigen T cell receptors*, an *immune synapse* forms between both types of cells and this is established through certain *cell adhesion proteins*

(4) This process activates the helper 2-T cells and stimulates them to secrete certain cytokines (= lymphokines) called *interleukins (ILs)* which stimulate differentiation & proliferation of B-lymphocytes (specially IL 4)

(5) The B lymphocytes secrete antibodies called *immunoglobulins* (figure 19) that attack and kill the invading antigens as described below.

In addition, the helper-2 T cells are activated by *IL-1* (secreted by the macrophages) and B cell differentiation is further stimulated by *IL-6* (secreted by both the macrophages and the helper-2 T cells).

Immunoglobulins (= Antibodies)

Immunoglobulins(Ig) include 5 main types termed **IgM** (75%), **IgA**, **IgG**, **IgD** and **IgE**. Each is formed of *4 polypeptide chains* (2 long heavy chains and 2 short light chains) that are joined by disulfide bridges (figure 20).

They protect the body against invading antigens by **2 ways** :

(1) **Direct inactivation of the invader** : This occurs by either :

(a) *Agglutination* (b) *Precipitation* (c) *Neutralization* (d) *Lysis* (e) *Opsonization (specially IgG)*.

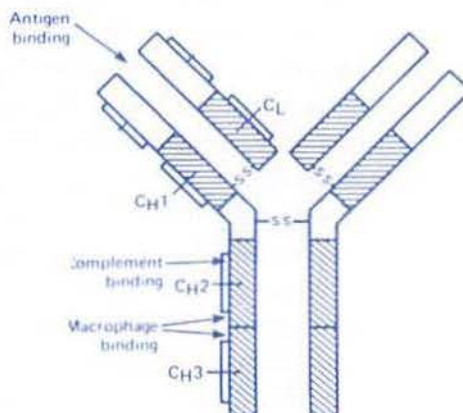


Figure 20 : A typical IgG molecule.

(2) **Activation of the complement system** : This is a system of *more than 30 specific proteins in the plasma*. These proteins are *inactive enzyme precursors* which become activated secondary to antigen-antibody reactions by *a cascade of reactions* that successively activate the components of the system. These reactions result in formation of *multiple end products* that help destruction of the invaders by the following mechanisms :

1. **Opsonization** (which enhances phagocytosis of bacteria).
2. **Agglutination** of the invading organisms.
3. **Neutralization** (specially of viruses).
4. **Chemotaxis of neutrophils and macrophages** to the site of the antigen-antibody reaction.
5. **Lysis** (certain end products cause insertion of *hole-forming proteins* called *perforins* into the membranes of bacteria or other invading organisms, which allow inward flow of ions leading to lysis of these invaders).
6. **Activation of mast cells and basophils** : These release histamine and other substances that lead to *local inflammatory effects* that help inactivation and immobilization of the invading antigen.

(B) CELLULAR (= CELL- MEDIATED) OR T- CELL IMMUNITY

This is produced by the *cytotoxic T- lymphocytes*, and is a major defence against *viruses, fungi and a few bacteria (e.g. the tubercle bacillus)* as well as against *tumour cells and foreign tissue transplants*. These lymphocytes *directly attack and destroy the antigen- containing cells* (figure 21) by inserting *perforins* (in the same way as the products of the complement system do) as well as by releasing certain toxic substances that *hasten cell apoptosis* (= death of the cell).

Mechanism of cellular immunity

The cytotoxic T-lymphocytes *cannot be activated by direct contact with the antigens*. They are activated by the following mechanism :

- (1) Antigens are engulfed by the *antigen presenting cells* (APCs).
- (2) In the APCs, the peptide products of antigen digestion are coupled to the *MHC-I protein* and are presented on the cell surface. *The CD8 present on the surface of the cytotoxic T- lymphocytes can bind to the MHC-I protein, so these cells will start attacking the APCs.*
- (3) Some APCs present the MHC-II on their surfaces. These cells come in contact with *CD4 helper-1 T cells* (since CD4 present on the surface of the helper-1 T cells can bind to the MHC-II protein) and an *immune synapse* forms between both types of cells.
- (4) This process activates the helper-1 T cells and stimulates them to secrete certain *interleukins* (specially *IL-2*) which further increases the activity of the cytotoxic T- lymphocytes.

****** the helper-1 T cells are also activated by *IL-12* secreted by the macrophages. In addition, *IL-2* autoactivates the helper-1 T cells in a positive feedback way.

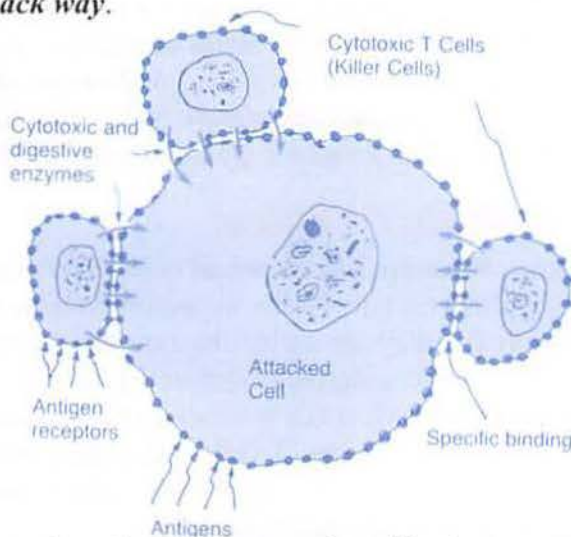


Figure 21 : Destruction of an antigen presenting cell by the cytotoxic T- lymphocytes.

The suppressor T cells : A 4th type of T lymphocytes (in addition to the helper, cytotoxic and memory T cells) that *help termination of the immune response* is suggested and is called the suppressor T cells. These cells were also claimed to *play a role in producing immune tolerance*. However, their presence is doubtful.

Passive immunity : Temporary immunity in an individual against a certain antigen can be achieved by giving him *already-formed antibodies or activated lymphocytes from another individual* who has been actively immunized against that antigen. This is known as *passive immunity* (in contrast to *active (acquired) immunity* that develops by activation of the immune system).

Role of the helper T lymphocytes in immunity : The helper T cells are the *most numerous type of the T cells* (about 75%) and they are the *major regulator of all immune functions*. Helper-2 T cells activate the B-cells by IL-4 and 6 while helper-1 T cells activate themselves as well as the cytotoxic T- cells by IL-2 (see above). Helper-1 T cells also release *gamma interferon* (see below).

The HIV (Human Immunodeficiency Virus) that causes the fatal disease called AIDS (= **Acquired Immune Deficiency Syndrome**) destroys the helper- T cells (leaving the body totally unprotected against infections).

Role of macrophages in immunity : The macrophages are the main *antigen presenting cells* which activate the T lymphocytes. They also *release ILs-1,6 and 12* which activate both the B and T lymphocytes.

THE NATURAL KILLER CELLS (NK CELLS)

These are a type of lymphocytes that are *non-T non-B lymphocytes*. Like the cytotoxic T cells, they directly attack and destroy malignant cells, viral-infected cells & foreign tissue transplants *without involvement of the MHC*. They also produce *gamma interferon*. Since the cytotoxic T cells are 2 types (page 61), then the *human body has 3 types of killer cells*.

THE LYMPHOKINES (CYTOKINES)

Lymphokines are *hormone-like chemical messengers* that are secreted not only by the lymphocytes but also by the *macrophages, neurons, glial and endothelial cells* (so they are called the *cytokines*). They affect the immune system and produce widespread systemic reactions. They include

(1) **Interleukins (ILs)** : Most ILs were discussed above. Some of the others include IL-5 (secreted by the T cells and causes differentiation of eosinophils) and IL-8 (secreted by the T cells and macrophages, and causes chemotaxis of neutrophils and basophils).

(2) **Interferons** : There are 3 types :

(a) *Alpha and beta interferons* (secreted by virally infected cells and it induce resistance of cells to viral infection).

(b) *Gamma interferon* (secreted by helper-1 T lymphocytes and NK cells) and they activate the macrophages.

(3) **Tumour necrosis factors (TNFs)** : There are 2 types, *alpha* (secreted by the macrophages, NK cells, all lymphocytes and mast cells) and *beta* which is also called *lymphotoxin* (secreted by B and helper-1 T lymphocytes). All these factors induce *promotion of inflammation*.

(4) **The granulocyte-macrophage colony-stimulating factor.**

TISSUE TRANSPLANTATION (GRAFTING)

The transplanted grafts or organs may be obtained from a donor of either the same species (= *allografts*) or a different species (= *xenografts*). However, both will be *rejected within a few weeks as a result of development of an immune response against them* (particularly the latter). The only grafts that are not rejected are those obtained from *identical twins* (= *isografts*) and the greater the genetic similarity between the recipient and the donor, the lesser the rejection would occur. Tissue rejection can be prevented by drugs that *depress T-cell activity* (e.g. azathioprine, cyclosporin-A, *tacrolimus* and large doses of glucocorticoids).

Tissue matching : This is a test that detects whether a tissue transplant will be rejected or not. The *lymphocytes of the donor* (which are rich in the *HLA* (page 62) are matched against the lymphocytes of the recipient. If no reaction occurs, the transplant will not be rejected.

DISEASES RELATED TO THE IMMUNE RESPONSE

(1) **Autoimmune diseases** (e.g. rheumatic fever, myasthenia gravis, glomerulonephritis, Graves' disease, type 1 diabetes mellitus, multiple sclerosis, lupus erythematosus, celiac sprue). These diseases occur as a result of failure of *self or immune tolerance* (page 61).

(2) **AIDS** (page 65).

(3) **Deficient immune response** : This occurs due to either:

a- Failure of the lymphocyte precursors to develop or to migrate to the thymus and liver *This abolishes both humoral and cellular immunity.*

b- Congenital absence of the thymus gland. *This abolishes the cellular immunity only.*

c- Failure of development of the B- lymphocytes. *This abolishes the humoral immunity only.*

(4) **Allergic diseases** : In these diseases, there are violent antigen-antibody reactions due to *abnormal hypersensitivity to certain antigens* called **allergens**. The patients have a *genetic tendency* and are characterized by presence of large amounts of **IgE**. Such antibodies are strongly attached to the **mast cells and basophils**, and their reaction with the allergen causes rupture of these cells which release the following :

(a) **Histamine and the slow reacting substance of anaphylaxis** (= a mixture of toxic substances called *leukotrienes*) which cause V.D. and increase the capillary permeability with loss of fluid into the tissues. They also cause **bronchial constriction**, which may be fatal due to suffocation.

(b) **A protease** which causes local tissue damage.

(c) **Eosinophilic and neutrophilic chemotactic substances.**

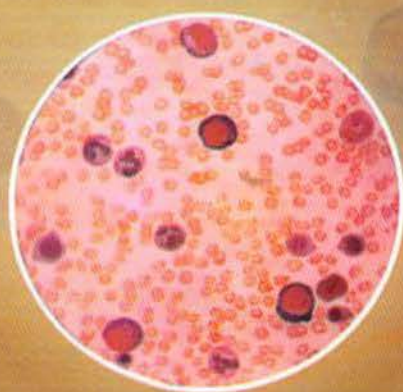
Widespread reactions may cause a fatal **anaphylactic shock**, but localized reactions produce other diseases e.g. **urticaria and rhinitis**. These conditions can be treated by (1) *Antihistaminic drugs* (2) *Norepinephrine*, which prevents hypotension (3) *Glucocorticoid hormones* (e.g. *cortisone*) which depress the immune response.

AUTHOR'S AVAILABLE BOOKS

1. Human physiology for medical students : **Autonomic nervous system & Nerve and Muscle.**
2. Human physiology for medical students : **Blood and body fluids.**
3. Human physiology for medical students : **Circulation (cardio-vascular system).**
4. Human physiology for medical students : **Respiration.**
5. Human physiology for medical students : **Digestion (GIT).**
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10. Human physiology for medical students : **Energy metabolism.**
11. **Oral and written questions and answers** (volume 1).
12. **Oral and written questions and answers** (volume 2).
13. **M.C.Q.s and answers** (volume 1).
14. **M.C.Q.s and answers** (volume 2).
15. A summarized guide to **circulation.**
16. A summarized guide to **CNS.**

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